# DEMOGRAPHY AND GENETIC DIVERSITY IN *TRADESCANTIA OCCIDENTALIS* (COMMELINACEAE)

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**ABSTRACT**

Genetic diversity has rarely been the focus of study in species at risk in Canada. *Tradescantia occidentalis* is one of 157 species listed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2005*a*). This species is nationally threatened due to the limited number of populations, their geographic isolation from each other and from the main distribution in the United States of America, and habitat loss. The National Recovery Team for Plants at Risk in the Prairie Provinces and the Alberta Western Spiderwort Recovery Team have called for research into the habitat requirements, demography, and genetic diversity of *T. occidentalis* in Canada. As a result, this study was designed to address the following objectives: 1) to conduct an inventory of the Canadian populations, 2) to investigate intra- and interpopulation genetic diversity in *Tradescantia occidentalis*, and 3) to provide recommendations for the conservation management of this species.

Information on demography and plant communities in *Tradescantia occidentalis* habitats indicated that the numbers of individuals in the Saskatchewan and Manitoba populations were similar to previous surveys; however, the Alberta population was significantly larger in number than prior estimates, indicating population growth. Taxonomic lists were prepared for each province in habitats with and without *T. occidentalis*. Community types, as separated by RA analysis, differed by province and not by association with *T. occidentalis*. *Euphorbia esula*, an invasive species in Canada, was observed in the Saskatchewan and Manitoba populations but was absent in Alberta.

Using amplified fragment length polymorphisms (AFLPs), genetic diversity was assessed at the intra- and interpopulation levels. Relatively low levels of intrapopulation

variation were observed in Saskatchewan and Alberta, while higher levels were found in Manitoba. Gene flow via pollen or propagule transfer may account for higher genetic diversity among the closely situated Manitoba populations. The lack of correlation between dendrogram topology and geographic distribution suggests panmixia in all populations. Levels of intrapopulation diversity were low to moderate depending on primer combination used, indicating that populations are isolated within each province.

Information on population demography and genetic diversity are important within a conservation context. The large number of individuals within each population and the perceived increase in some populations suggest that the existing populations of *Tradescantia occidentalis* are relatively stable. Although levels of genetic diversity are low in Saskatchewan and Alberta compared to Manitoba, it appears that all populations are adapted to their local environments based on their apparent size and stability. The most viable conservation strategy for this species is *in situ* protection. This should include controlling invasive plant species, monitoring grazing, and preventing further habitat fragmentation. *Ex situ* methods must also be explored. Transplantation of individuals from one population to the next may not be a successful conservation strategy due to the moderate level of population differentiation. Alternatively, it is recommended that a seed bank from each population be implemented in case of a drastic population decline.

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## LIST OF ABBREVIATIONS

ASRD: Alberta Sustainable Resource Development.

bp: Base pair.

CA: Correspondence analysis.

COSEWIC: Committee on the Status of Endangered Wildlife in Canada.

CTAB: 2X hexacetyl trimethylammonium bromide.

$dH_20$: Distilled water.

dNTPs: Deoxynucleotide triphosphates.

MCDC: Manitoba Conservation Data Center.

MHHC: Manitoba Habitat Heritage Corporation.

NJOIN: Neighbour joining method

NTSYS: Numerical Taxonomy System software program.

PC-ORD: PC-ORD Multivariate Analysis of Ecological Data software program.

PCOORDA: Principle coordinate analysis.

PCR: Polymerase chain reaction.

PFRA: Prairie Farm Rehabilitation Association.

RA: Reciprocal averaging.

RAPD: Random amplified polymorphic DNA.

*rbc*L: Chloroplast gene coding for the large subunit of ribulse-1,5-bisphosphate carboxylase.

RFLP: Restriction fragment length polymorphisms.

SAHN: Sequential, agglomerative, hierarchical, and nested clustering methods.

SASK: Herbarium of the University of Saskatchewan.

SERM: Saskatchewan Resource and Environment Management.

SSR: Single sequence repeat.

UPGMA: Unweighted pair group method with arithmetic mean.

## 1. INTRODUCTION

There are over 150 species officially designated as at risk in Canada, several of which are understudied. Despite the growing number of endangered taxa of flora and fauna, little is known about the biology of most of these vanishing species. *Tradescantia occidentalis* (western spiderwort), a member of the Commelinaceae family, is among these species at risk. Conservation of plants at risk requires a holistic approach involving numerous areas of study. The Alberta Western Spiderwort Recovery Team and the National Recovery Team for Plants at Risk in the Prairie Provinces have called for the investigation of several aspects of the biology of *T. occidentalis*, including reproductive biology and habitat requirements. In addition, these organizations have acknowledged a paucity of information regarding levels of genetic diversity in this species.

Terrestrial plants are susceptible to changes in habitat because of their sessile nature. As a result, it is important to investigate macro- and micro-ecological variables including soil conditions, water and nutrient availability, associated plant communities, presence of invasive plant species, and effects of grazing. These parameters, along with consistent methods to estimate population size, must be addressed to preserve habitat to sustain species at risk.

The integration of ecological and molecular techniques in a conservation context is becoming popular in studies on rare and endangered species to address issues in a more holistic manner. Molecular techniques are used to assess levels of genetic

variation at the intra- and interpopulation levels and are usually used in conjunction with ecological data. Issues such as inbreeding depression and the resulting loss of fitness should be addressed in relation to environmental variables and isolation of populations. Although it is unclear what levels of intrapopulation diversity are required to prevent a loss of adaptability through inbreeding depression, these values can be compared among populations of the same species. The level of interpopulation differentiation is an important consideration in designing conservation strategies, such as transplantation of individuals among populations.

To date, genetic variation has not been widely explored in threatened species in Canada and this project represents one of the first of this kind. This study is comprised of two main parts: 1) population demography, and 2) genetic diversity. Conducting population inventories was not initially proposed in the project design, but was required in some provinces in order to obtain permission to access and collect material from *Tradescantia occidentalis*. Thus, though it was not intended to be a complete ecological study, this research provides fundamental information regarding population size as part of an annual assessment. The main focus of this research is the estimation of levels of genetic diversity in Canadian populations of *T. occidentalis*. Each thesis chapter is written in the format of a paper to be submitted to a peer reviewed journal; the ecology chapter to *Canadian Field Naturalist* and the molecular chapter to *Genome*. Taxonomic authorities for plant names are provided in Appendices II and III where they are not provided in the text. The information gathered in this study will enhance the species based conservation strategy already in place for *T. occidentalis* and may play a role in formulating a large scale conservation plan for sand dune habitats. This study will also serve as a model for future studies of species at risk in Canada and around the globe.

## 2. LITERATURE REVIEW

### 2.1 The Commelinaceae

The Commelinaceae is a monocotyledonous plant family that occurs in pantropic and warm temperate climates. It encompasses 40 to 50 genera and approximately 600 species (Tucker 1989; Evans et al. 2000), some of which are considered invasive (Faden 1993; Standish 2001). Only four species of this family, placed in the genera *Tradescantia* L. and *Commelina* L., are native to Canada. Although there is disagreement in the taxonomic treatments of the Commelinaceae, it is divided into two subfamilies, the Cartonematoideae and the Commelinoideae. This separation is based on the distribution of raphide canals and the presence or absence of glandular microhairs (Faden and Hunt 1991). Each subfamily is divided into two tribes, the Cartonemateae and Triceratellae and the Tradescantieae and Commelinae, respectively. This division of the Commelinoideae is not supported by cladistic analysis of morphological and anatomical characters (Evans et al. 2000); however the monophyly of these two tribes is supported by molecular data of the *rbc*L gene (Evans et al. 2003).

Genera of the tribe Tradescantieae are described as erect to prostrate plants with monopodial stems (Woodson 1942). The inflorescences consist of sessile, terminal or axillary, paired cincinni (scorpioid cymes) subtended by conspicuous bracts (Woodson 1942; Brenan 1966). These two-sided cincinni are considered to be derived from the individual cymes of the Commelinae (Woodson 1942). In the Tradescantieae, the

actinomorphic flowers have six fertile stamens and produce seeds with a linear or punctiform hilum (Brenan 1966). In contrast to the Tradescantieae, members of the Commelinae have zygomorphic flowers in one-sided, scorpioid cymes (Woodson 1942). The tribes can be further divided based on morphological characters. For example, based on root anatomy the Tradescantieae is split into seven subtribes, three of which occur in the New World (Hofreiter and Tillich 2002).

Several morphological features distinguish the Commelinaceae from the other commelinoid monocots, including an amoeboid tapetum (Evans et al. 2000), closed leaf sheaths, and succulent leaf blades (Faden and Hunt 1991). Furthermore, the presence of cleistogamous flowers is a unifying feature in some species of the Commelinaceae (Faden 2000). In several species these flowers are subterranean and produced from nodes of decumbent shoots (Faden 1993). The aerial flowers have green sepals, coloured petals, and three to six fertile stamens with bearded filaments in many genera. The tetrasporangiate anthers, with distinctive girdle-type wall thickenings, dehisce introrsely to release binucleate, monosulcate pollen grains (Tucker 1989). Axillary placentation and orthotropous ovules are characteristic of the syncarpous, trilocular ovary in most genera of this family. In the Commelinaceae, seeds with an abundance of starchy endosperm are produced in capsules, nutlets, or berries (Tucker 1989). Seeds are light and weigh approximately 0.003 g each (Stevens 1932).

Some particular chemical compounds also characterize the Commelinaceae. In a number of genera, including *Coleotrype* C. B. Clarke, *Commelina*, *Gibasis* Raf., *Tradescantia*, and *Tripogandra* Raf., sulfated phenolic acids, quercetin 3-glycoside, calcium oxalate raphides, and steroidal saponins are present (Tucker 1989). The

anthocyanins commelinin (p-coumaroyl-delphinidin 3,5 glucoside) and cyanidin (3,7,3'-triglucoside) are responsible for the characteristic blue petal colour in this family (Tucker 1989). Additional anthocyanins known as acylated glucosides have not been reported in other monocots and appear to be unique to this family (Tucker 1989).

The Commelinaceae have limited commercial use but they are culturally important for several reasons. Ornamental species, such as *Tradescantia*, *Commelina*, and *Palisota* Rchb. ex Endl., are cultivated for gardens or as house plants. The ornamental use of these plants can be problematic because several species are considered invasive, particularly in areas of the United States of America and New Zealand (Standish 2001). These invasive species have higher biomass and rates of sexual (Burns 2004) and vegetative reproduction (Faden 1993), resulting in higher fitness (Burns 2004) compared to non-invasive species.

In addition to ornamental uses, species in the Commelinaceae can be used for food or medicine. The starchy tuberous roots of the genus *Murdannia* Royle and some *Commelina* species are edible (Tucker 1989). Yanovsky (1936, reviewed in Tucker 1989) reports that young shoots of *Tradescantia occidentalis* were eaten by the Great Plains Indians. Furthermore, certain species have been used for their medicinal properties. For example, *Commelina virginica* L. has been reportedly used for clotting blood in surface wounds, as well as in curing ailments of the stomach (Herrera 1897, reviewed in Tucker 1989). *C. nudiflora* L. leaves are crushed and applied to the forehead to relieve fever by Kadazandusun communities in Malaysia (Ahmed and Ismail 2003), while in Tanzania, *C. benghalensis* L. is used in the preparation of a vaccine against blackquarter (Minja 1999). In addition, *C. diffusa* Burm. f. is used to treat fevers

and leucorrhea and in detoxification of the body (Long and Li 2004). There are numerous other examples of medicinal uses of the Commelinaceae, but in spite of their therapeutic properties, the chemically active components of the family have not been characterized.

### 2.1.1 Root System

Root morphology and anatomy has been widely studied in the Commelinaceae. Different root types have been observed in the family, for example *Tradescantia hirsuticaulis* Small has drop roots with rootlets (Pinkerton 1936), whereas other species have fibrous roots (Faden 2000). Generally, Commelinaceae roots are fleshy, succulent, and shallow, enabling these plants to compete with grasses for water and nutrients through lateral spread (Weaver 1958). The roots commonly reach a depth of 1.5 to 3 ft and spread horizontally up to 1.5 ft, which provides the greatest competitive advantage in dry climates with light summer rain (Weaver 1958) or in other climates with limited moisture. Some members of this family, for example *T. virginiana* L., are associated with arbuscular mycorrhizal fungi (DaSilva et al. 2001), which may confer advantages for resource acquisition.

The root anatomy of the Commelinaceae is quite varied and the characters are not phylogenetically informative (Hofreiter and Tillich 2002); therefore only the anatomy of the Tradescantiinae is provided here. In this subtribe, the exodermis consists of one layer of cells with evenly thickened walls. The pericycle lacks lignified cells and sclerenchyma (Hofreiter and Tillich 2002). Within this subtribe, the roots of

*Tradescantia virginiana* are unique because they are bistelic (Hofreiter and Tillich 2002).

Two types of secondary root hairs with thickened cell walls, i.e. transitory and persistent hairs with dead, air-filled cells, have been observed in the Commelinaceae (Pinkerton 1936). There is a correlation between the persistent hairs and the absence of secondary growth of roots in this family, which is thought to result in increased absorptive efficiency and anchorage in shallow root systems (Pinkerton 1936). Absorption and anchorage are particularly important for species, such as *Tradescantia occidentalis*, that are found in sandy, unstable soils. Additional aspects dealing with root morphology in *T. occidentalis* are discussed later in this study in relation to ecological conditions.

### 2.1.2 Cytology

Knowledge of chromosome numbers is important because it relates to the reproductive biology of a plant group. The basic chromosome number in the Commelinaceae ranges from x=6 (2x=2n=12) to as high as x=20 (Jones and Jopling 1972). Generally the New World Commelinaceae are x=6 or x=8, while in Central and South America, the most common base chromosome numbers are x=17 and x=19 (Jones and Jopling 1972). Both diploids and polyploids, most commonly in the form of tetraploids, occur in the Commelinaceae (Anderson and Sax 1936). Tetraploids have a longer blooming period and higher survival rates than diploids but are generally only distinguished cytologically (Anderson and Sax 1936). Additional DNA in polyploids may be related to their ability to withstand several factors including temperature,

rainfall, UV intensity, latitude, altitude, and longer life cycle, and is considered adaptive when it coincides with morphological or ecological diversity (Kenton 1984). In several species, including *Gibasis linearis* Rohw. (Kenton 1984) and *Tradescantia occidentalis* (Anderson and Sax 1936), DNA content varies within and among populations. In fact, diploid and tetraploid *T. occidentalis* have been observed growing within a few feet of each other in Texas, U.S.A. (Anderson 1954). In the genus *Tradescantia*, diploid individuals have six pairs of large chromosomes (2n=2x-12), while tetraploids have 12 (2n=2x=24) (Anderson and Sax 1936).

Pollen fertility is generally high in both diploids and tetraploids. For example, in diploid *Tradescantia occidentalis*, 94% of pollen was fertile, while 89% pollen fertility was observed in tetraploids (Anderson and Sax 1936). High fertility of tetraploid pollen was also observed in *Rhoeo discolor* (Sw.) Stear, a close relative of *Tradescantia*, where tetraploids produced twice as much fertile pollen as diploids (Walters and Gerstel 1948). Due to the effect of temperature on chromosome pairing, pollen fertility in some *Tradescantia* species may be higher in periods of warm weather than after a series of cold, wet days (Anderson and Sax 1936).

### 2.1.3 Reproductive Biology

The reproductive biology of *Tradescantia occidentalis* has not been studied specifically; however, there is a plethora of information available on reproductive biology of the Commelinaceae. Though the pollen is small enough to be transported by wind (0.036 mm x 0.021 mm in *T. occidentalis*), it has been demonstrated that wind is not a major vector for pollen dispersal in this family (Sinclair 1968). Members of the

Commelinaceae are entomophilous or autogamous, and the main visitors to flowers are social and solitary bees and syrphid flies (Faden 1992). The honeybee (*Apis mellifera* L.) has been commonly noted to contact anthers and stigmas on *Tradescantia* flowers and has been observed to visit up to 73 flowers consecutively (Sinclair 1968). Weak odours have been reported in Commelinaceae flowers, in particular in *Tradescantia*, but these are not thought to be overly important in attracting pollinators (Faden 1992).

Members of the Commelinaceae lack nectar rewards and therefore must attract pollinators by alternative means (Evans et al. 2003). Thus, flower morphology is important. The absence of nectar has two major implications: 1) that flowers rarely attract a wide array of pollinators, and 2) that pollen must function in fertilization in addition to rewarding the pollinator (Faden 1992). Yellow-tipped trichomes on upper stamens mimic pollen and lure insects to flowers in the genus *Tinantia* Scheidw. (Simpson et al. 1986). In this commelinoid genus, the upper stamen produces only sterile food pollen and while insects gather this pollen, fertile pollen from the lower stamens is transferred to their bodies for pollination (Simpson et al. 1986). Anther dimorphism has also been documented in other species in the Commelinaceae. For example, three stamen types have been observed in *Commelina coelestis* Willd., including: 1) two cryptically coloured lateral stamens with copious amounts of pollen for cross pollination, 2) a cryptically coloured central stamen for pollinator reward and delayed autogamy, and 3) three bright yellow staminodes with small quantities of inferior pollen for insect attraction (Hrycan and Davis 2005).

Finally, staminal hairs may be important in pollination for several reasons. First, these showy structures may attract insects to the flower (Faden 1992). Second, filament

hairs may influence insect behaviour, i.e. the way an insect moves within the flower and how it collects pollen. For example, honeybees gather filaments together to collect pollen, a process that is impeded by filament hairs (Faden 1992). Additional functions of the filament hairs may be to retain pollen that is dislodged from the anthers or provide a foothold for small insects that must land to feed (Faden 1992).

The morphology of the stigma is also important in plant reproduction. In the Commelinaceae, trifid and triangular shaped stigmas are the most common types, though brush-like and circular stigmas are also found (Owens and Kimmins 1981). Almost all pollinating surfaces are 'wet' in this family (Owens and McGrath 1984), except in *Tradescantia*, which has a 'dry' stigmatic surface (Owens and Kimmins 1981). 'Wet' stigmas are thought to have better pollen attachment, hydration, germination, and pollen tube growth (Owens and Horsfield 1982). In addition, the papillate nature of the stigma surface may be involved in pollen germination. Papillae of the stigma degenerate rapidly in open flowers and at anthesis these structures may collapse (Owens and Horsfield 1982). The permeability of the papillae is greatest at the mid-region of the cell and no germination occurs at the tip (Owens and McGrath 1984).

In addition to flower morphology, breeding systems are an important aspect of reproductive biology, but remain poorly understood in many plant families. Some species in the Commelinaceae are facultatively autogamous (Hrycan and Davis 2005), while others are self-incompatible. The general rule in *Tradescantia* is self sterility (Sinclair 1968). In self-compatible species, temporal and spatial isolation of the stigma from the pollen may prevent inbreeding (Owens 1981). In a survey of 110 species in the

Commelinaceae, 55 were self-incompatible, 50 were self-compatible, and five had both breeding systems (Owens 1981).

Self-incompatibility is found primarily in species with actinomorphic flowers, such as those observed in the Tradescantieae; however self-compatible individuals are found in the Parasetcreasea and Tradescantia sections of the genus *Tradescantia* within this tribe (Owens 1981). In self-compatible species, autogamy is evident by the presence of large bracts subtending the inflorescence, which makes the flowers inconspicuous (Owens 1981). Conversely, large showy flowers and long styles encourage outcrossing even if the species is self-compatible (Owens 1981). Longer styles and showy flowers are two attributes of *Tradescantia occidentalis* that promote outcrossing in this species.

Self-incompatibility is the inability of the plant to produce seeds from the union of functional male and female gametes when self pollinated (Brewbaker 1957). In angiosperms, self-incompatibility is controlled by a single S gene locus with multiple alleles, which results in the inhibition of pollen germination or pollen tube growth (Brewbaker 1957; Owens and Kimmins 1981). The site of inhibition depends on the time of the production of incompatibility substances (Pandey 1960). There are two types of control over self-incompatibility, sporophytic and gametophytic, and no plant family has both self-incompatibility systems (Pandey 1960). Sporophytic self-incompatibility is found in plants with trinucleate pollen and the behaviour of the pollen is determined by the maternal genotype (Brewbaker 1957). In this self-incompatibility system, the S allele action is before cytokinesis; therefore, the incompatibility reaction is initiated on contact of the pollens grains to the stigmatic surface (Pandey 1960).

In gametophytic self-incompatibility, the inhibition occurs at some stage in pollen tube growth and is found in species with binucleate pollen (Annerstedt and Lundquist 1967). With this system, the germinating pollen grains are unable to penetrate the stigma (Pandey 1960). In gametophytic self-incompatibility, the S allele action occurs after cytokinesis and inhibiting substances are produced within each microspore, stopping tube growth in germinated pollen (Pandey 1960). In the Commelinaceae, the gametophytic self-incompatibility system has been observed in several species, including *Tradescantia virginiana* (Brewbaker 1957). In tetraploids, S allele interactions (competition or dominance) occur in the pollen grain (Brewbaker 1957). However, the lack of breakdown of incompatibility in tetraploids of the Commelinaceae indicates no allele competition (Annerstedt and Lundquist 1967). Additionally, partial breakdown in some polyploid *Tradescantia* species has been observed to have no effect on the self-incompatibility system (Owens 1981).

### 2.1.4 Interspecific Hybridization

Interspecific hybridization in the Commelinaceae is a common event (Kenton 1984). Interspecific hybridization often results in an increase in the percentage of sterile pollen (Anderson and Sax 1936). Although *Tradescantia occidentalis* (2n=2x=12) and *T. reflexa* Raf. (2n=2x=12) are known to hybridize in the American Midwest producing tetraploid offspring (2n=4x=24) (Anderson and Sax 1936), there are many barriers to interspecific hybridization, including habitat preference. Species in the Commelinaceae exploit different niches, for example rock outcrops, sand dunes, and forests, which may prevent hybridization (Anderson and Sax 1936). Isolation mechanisms that maintain

species integrity include the short time that the flowers are open, the carrying in of the stigmas and styles by deliquescing petals, and time of flowering (Sinclair 1968). Flowers in *Tradescantia* species in the United States have been reported to last only one day, opening around 5 AM and closing around noon (Sinclair 1968; Faden 1992).

### 2.2. The Genus *Tradescantia*

The genus *Tradescantia* includes approximately 70 species distributed in neotemperate and neotropical regions (Faden 2000). The placement of species within this genus has been widely debated and it is often divided into sections. For example, based on root anatomy, *Tradescantia* is divided into the following six sections: Tradescantia, Austrotradescantia, Setcreasea, Rhoeo, Campelia, and Zebrina (Hofreiter and Tillich 2002). These herbaceous, semi-succulent perennials have thin or tuberous roots that are covered in a piliferous layer (Pinkerton 1936). The linear, sessile leaves are spirally arranged along the stem (Faden 2000). The flowers are clustered in terminal or axillary, unilateral scorpioid cymes (cincinni) and are subtended by long, spathaceous bracts, which often extend beyond the inflorescence (Tucker 1989). The bisexual flowers are white to pink, blue, or violet with distinct petals and sepals (Faden 2000). The androecium has six showy stamens with bright yellow anthers and bearded filaments. The trilocular, syncarpous ovary forms a capsule with two to six seeds (Faden 2000). Stevens (1932) reported the magnitude of seed production in *T. occidentalis* to be in the order of 1,160 seeds in a mature plant; however these results may have been exaggerated by his estimation methods.

Only three *Tradescantia* species are found in Canada, namely, *T. ohiensis* Raf. and *T. virginiana* in southern Ontario, and *T. occidentalis* in the prairie provinces, the latter being the focus of the research presented here. In the United States of America, these species are much more common and *T. occidentalis* is actually considered as an invasive plant in some places (Anderson and Sax 1936; Anderson 1954). This species can survive in a variety of habitats including sandy terraces, mesquite forests, and grasslands (Anderson 1954), but in Canada it is restricted to five populations located in sandy habitats as described in the following section.

### 2.2.1 Morphology, Habitat, and Distribution of *Tradescantia occidentalis* in Canada

*Tradescantia occidentalis* (Fig. 2.1A) is a perennial monocot native to temperate regions of Canada and the United States of America. Injured stems and leaves produce a mucilaginous substance that resembles a cobweb when hardened, giving rise to the common name, western spiderwort. The fleshy, semi-succulent roots are an adaptation to the dry environment where this plant lives (Fig 2.1B). Flowers are clustered in cincinni and have three sepals, three pink or blue petals, and an androecium with bearded filaments and bright yellow anthers (Fig. 2.1C). The fruit is a capsule, each of which releases two to six seeds that are dispersed down slope of the parent plant by gravity, rain, snowmelt, or wind (Smith 2002). Methods of long distance seed dispersal are not known for this species, and local dispersal results in a clumped distribution of plants within a population. Seed set is very important to *T. occidentalis* because there are no methods of vegetative reproduction in this species. Although various insects have been observed visiting *T. occidentalis* flowers, sweatbees are the only confirmed

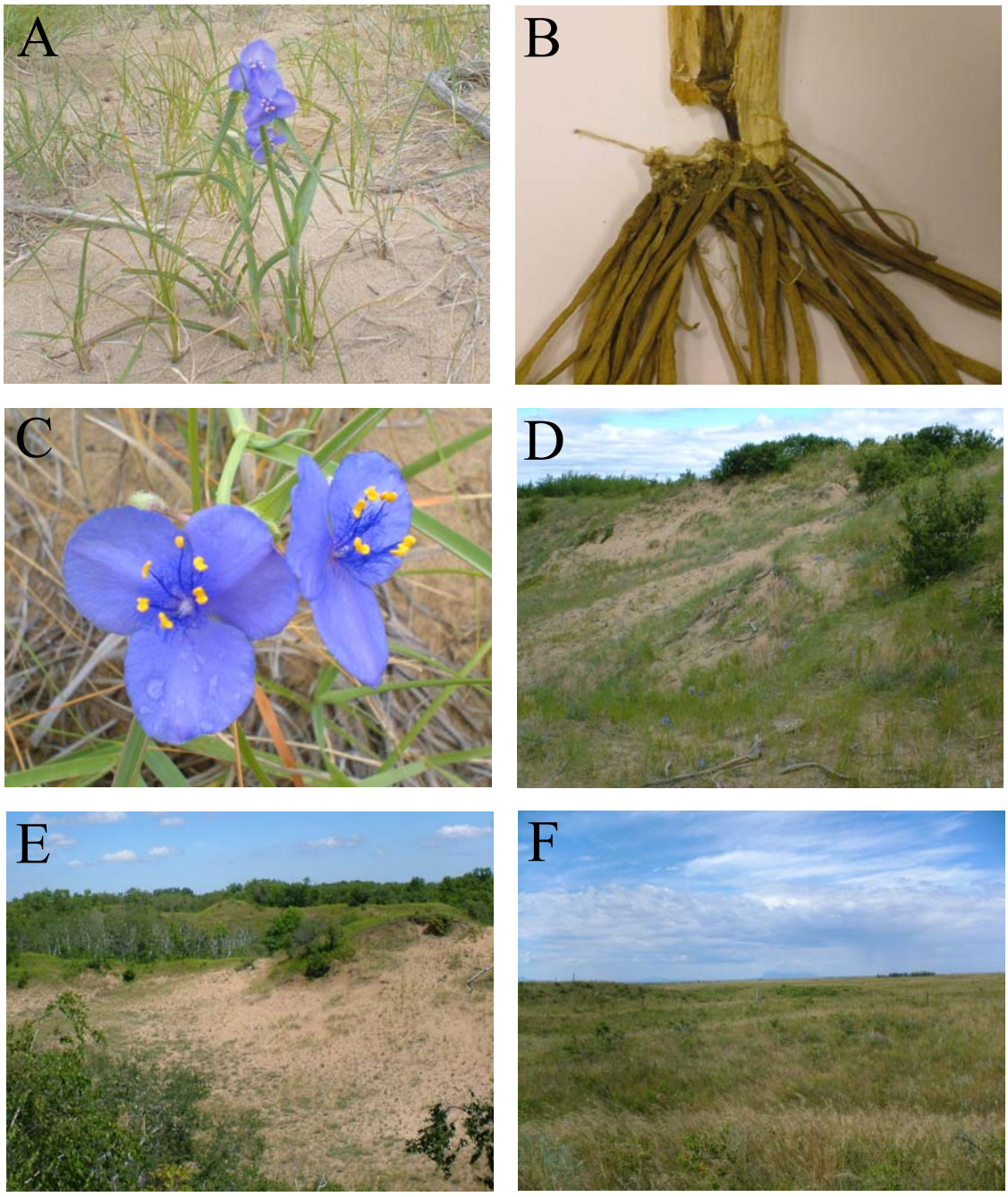


**Figure 2.1.** Plant, roots, flowering structures, and habitats of *Tradescantia occidentalis*. **A.** Plant with linear, sheathing leaves. **B.** Fleshy semi-succulent roots. **C.** Flowers with bearded filaments. **D.** General habitat in the PFRA subset of the Saskatchewan population. **E.** General habitat in the Lauder (Manitoba) population. **F.** Rolling stabilized dune habitat in Alberta.

pollinators of this species (Smith 2002).

*Tradescantia occidentalis* was studied in two types of habitat in Canada. First, this species is locally abundant on sand dunes (Fig. 2.1D, E), excluding areas dominated by *Populus tremuloides* or *Quercus macrocarpa*. Plants in this type of habitat are primarily located midslope on the southwest face of partially stabilized dunes (Godwin and Thorpe 2004; MCDC 2005). In Saskatchewan, this species occurs mainly in a rolling sand dune complex (Fig. 2.1D) with smaller clusters located in relatively flat sandy areas with moderate vegetation. In Manitoba, *T. occidentalis* populations are found on discrete sand hill formations and occasionally in small shaded meadows or among low growing shrubs (Fig. 2.1E) (Goulet and Kenkel 1997). These sand hill formations are linear or parabolic in shape with slopes generally greater than 30˚ (MCDC 2005). This plant occasionally grows in dense, shrubby or wooded vegetation but prefers sunny areas associated with open sand or sparse vegetation in this environment. Because this species has several adaptations to drought conditions, including fleshy roots, rubbery leaves, mucilage, and flowers that close in midday heat, it is better suited to the warm dry conditions of the partially active dunes than the moist shady environment typical of wooded communities. Second, in contrast to the Saskatchewan and Manitoba populations, very few individuals of *T. occidentalis* are found in active sand dunes in Alberta. The vast majority of this population occurs on sandy substrate that has been stabilized by vegetation. In this habitat, the dunes are eroded and only small, undulating stabilized hills remain (Fig. 2.1F).

Though *Tradescantia occidentalis* is adapted to different environmental conditions across the prairies, the habitats have shared features, such as sandy soil and several plant

species. According to Peters (2003*a*), *T. occidentalis* in Alberta is associated with the following species: *Hesperostipa comata* subsp. *comata*, *Calamovilfa longifolia*, *Koeleria macrantha*, *Bouteloua gracilis*, *Rosa* L., *Lygodesmia*, and *Rumex venosus*. *T. occidentalis* has also been observed with *Symphoricarpos occidentalis*, *Prunus virginiana*, *Elaeagnus commutata*, *Selaginella densa*, and *Artemisia ludoviciana* (Smith 2001).

### 2.3 Species at Risk in Canada

*Tradescantia occidentalis* is among a large number of plant species in need of protection. At present, there are 157 vascular plants on Canada's species at risk list, of which 74 are listed as endangered, 48 as threatened, and 35 in the special concern category (COSEWIC 2005*a*). Several of these species are in danger of extirpation due to habitat specificity, fragmentation or destruction, and competition with invasive plants.

While threatened species are in less immediate danger of extirpation than endangered species, research must be conducted to better understand all species at risk. *Phacelia ramosissima* Dougl. ex Lehm., *Epilobium densiflorum* (Lindl.) Hoch & P.H. Raven, and *Meconella oregana* Nutt. are examples of species in the endangered category (COSEWIC 2005*a*). *P. ramosissima* is restricted to three populations in Canada, each with less than 1,000 plants, and is in the endangered category due to habitat loss as a result of urban expansion and mining activities (COSEWIC 2005*b*). Likewise, there has been a decrease in the number of populations of *E. densiflorum* due to reduction of the natural habitat for agricultural, urban, and industrial development, with subsequent spread of exotic species (COSEWIC 2005*d*). A similar situation has

been observed in *M. oregana* where the 3,500 mature individuals that remain in Canada are threatened by overgrazing, fire suppression, and the influence of invasive taxa (COSEWIC 2005*f*).

Other species, including *Tradescantia occidentalis*, *Castilleja rupicola* Piper ex Fern., and *Enemion biternatum* Raf., are among the taxa in the threatened category (COSEWIC 2005*a*). *C. rupicola* populations are highly fragmented, geographically restricted, and are estimated consist of less than 250 individuals (COSEWIC 2005*c*). *E. biternatum* is affected by a reduction in the quality of its remaining habitat caused by recreational activity, soil compaction, trampling, and erosion, and by the use of chemicals, such as pesticides and road salt (COSEWIC 2005*e*). *Cypripedium reginae* Walt. and *Polygonatum biflorum* (Walt.) Ell. are not ranked by COSEWIC, but are considered provincially endangered in Saskatchewan (Harms 2003); thus, they must be monitored to preserve provincial biodiversity. These taxa are also affected by loss of habitat and an increasing number of invasive species.

In spite of the large number of species at risk in Canada and the implications of human activity on biodiversity, the information regarding the general biology, population size, and genetic structure and diversity is lacking or outdated for many species. It is clear that numerous species are in need of investigation because scientific knowledge is required to implement effective conservation management strategies. Reproductive biology, response to invasive taxa, habitat requirements, and genetic diversity are a few examples of understudied areas. An emerging tool in conservation is the use of molecular techniques to assess genetic diversity and population structure of species at risk. Studies of genetic variation in species at risk using molecular markers

have been increasing due to their fundamental role in conservation and because they provide an effective and relatively quick assessment of population genetics.

### 2.3.1 COSEWIC Status of *Tradescantia occidentalis*

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) lists and prioritizes species of flora and fauna according to their degree of risk. Several issues are taken into account when placing species in a particular category. For example, a species may be considered endangered when the decline in population number is greater than 70% of the total population or the number of individuals is less than 2,500 in Canada (COSEWIC 2005*a*). Likewise, a species may be considered threatened if the decrease in the number of individuals is greater than 50% or the total number of individuals is less than 10,000 (COSEWIC 2005*a*).

*Tradescantia occidentalis* is nationally threatened (COSEWIC 2005*a*). On a provincial scale, this species has been ranked as endangered in Saskatchewan (Wild Species at Risk Regulations 2005) and Alberta (AESCC 2005), and as threatened in Manitoba (MCDC 2005). The COSEWIC designation of this species is based on several factors, including the limited number of populations and restricted distribution in Canada. *T. occidentalis* occurs in only five populations in Canada: one in Saskatchewan, one in Alberta, and three in Manitoba. These populations are isolated among the provinces but in the United States of America if forms a continuous distribution. This species is at the northernmost extent of its range, explaining in part its inability to expand northward. However, there are several geographically proximal dune complexes or sandy areas of apparently suitable habitat unoccupied by *T.*

*occidentalis* to the south, east, and west of current populations, indicating that there may be other factors preventing a wider distribution of this species. Additional available habitat is located in other areas of the Douglas Provincial Park, the Great Sand Hills, and the Webb Sand Hills in Saskatchewan (K. Remarchuk, pers. obs.) and at Pakowki Lake South, One-Four, Turin, Purple Hills, Lazy H, Hilda and Whiskey Gap in Alberta (Peters 2003*a*, *b*), but *T. occidentalis* has not been located in these regions. Barriers to seed dispersal and differences in microhabitat or ecological conditions may limit the dispersion of *T. occidentalis* to new areas.

In addition to the limited number of populations, it has been reported that the number of individuals within a population fluctuates from year to year (Smith 2002), though discrepancies among studies may be due to different inventory methodologies or sampling error. For example, in 2003 and 2004, the large portion of the Saskatchewan population residing on Prairie Farm Rehabilitation Association (PFRA) land was estimated at approximately 40,000 and 60,000 individuals, respectively (Godwin and Thorpe 2004, 2005). In 2004, the two small Saskatchewan subpopulations (Douglas and Highway 19) were estimated at 2,137 and 758 stems, respectively (SERM 2005). However, in 2001, the total Saskatchewan population was estimated to be a meager 100 individuals (Smith 2002). These discordances are partly due to the discovery of additional patches of *T. occidentalis* in the PFRA pasture and in the Douglas Provincial Park in subsequent years. A similar discrepancy has been observed in the Manitoba and Alberta populations. In addition to reported demographic fluctuations, habitat destruction and fragmentation, and competition with invasive plants also pose a threat to

this species (Peters 2003*a*). Furthermore, overgrazing may affect population numbers and hence the status of *Tradescantia occidentalis*.

### 2.4 Measurement of Genetic Diversity as a Conservation Tool

The need for studies to document biodiversity and propose management strategies for plant species at risk is critical. The use of molecular techniques to infer levels of genetic diversity is becoming widely used in rare and endangered plants for conservation management purposes (Godt et al. 1995; Cardoso et al. 1998; Camacho and Liston 2001). Knowledge of genetic diversity provides insight into speciation, adaptation, and population structure and dynamics (Bussell 1999). In addition, the screening of genetic variability in endangered species provides valuable baseline data for conservation strategies such as new and more efficient sampling strategies, reintroduction of extirpated or endangered plants, translocations, and the introduction of new genotypes to increase genetic diversity (Arafeh et al. 2002). Furthermore, the identification of genetically diverse populations as a source of propagules may be useful (Godt et al. 1995).

#### 2.4.1 Molecular Methods for Estimating Genetic Diversity

A number of methods have been proposed to estimate levels of genetic diversity. To date, there is disagreement regarding the best molecular method to determine genetic variation, and the choice often depends on the organism under study and the methods available. Molecular fingerprinting provides insight into genetic structure, divergence, and phylogeny of organisms (Martínez-Ortega et al. 2004) and involves the use of

genetic markers, which are heritable polymorphisms that reflect differences in nucleotide sequences (Schubert et al. 2001). Numerous molecular methods have been explored to investigate genetic diversity, including amplified fragment length polymorphisms (AFLPs) (Mace 1999), restriction fragment length polymorphisms (RFLPs) (Powell et al. 1996), simple sequence repeat (SSR) polymorphisms (Powell et al. 1996; Mariette et al. 2001), and random amplified polymorphic DNA (RAPD) (Ayres and Ryan 1999). Each of these methods has advantages and disadvantages. For example, the SSR technique is ideal for studies among closely related species or within species because it provides high resolution for detecting heterogeneity and heterozygosity, but for the same reason is not useful at higher taxonomic levels, such as among genera (Powell et al. 1996). RFLPs have a high multiplex ratio, meaning that a large amount of information is generated in each experiment, but do not distinguish heterozygotes from homozygotes (Powell et al. 1996).

AFLPs are commonly used in studies at the population level. Overall, the amount of information obtained with AFLPs exceeds that of RAPDs by a factor of ten (Koopman 2005). AFLPs are useful in calculating genetic distances between genotypes (Karp et al. 1996) because polymorphisms made visible when DNA from two sources differ in restriction sites are detected by this technique (Hongtrakul et al. 1997). Also, there are fewer problems with reproducibility relative to other techniques because longer primers and higher annealing temperatures can be used, thereby increasing selectivity through more stringent conditions (Nybom 2004). In general, techniques that involve restriction enzyme digestion and two polymerase chain reaction (PCR) primers are more robust and reproducible than other methods (Karp et al. 1996). Two restriction enzymes

are used to prevent doublets due to unequal mobility by labeling one strand of PCR products (Vos et al. 1995). Furthermore, AFLPs span a large region of the genome without requiring prior sequence knowledge (Russell et al 1999; Deprés et al. 2003). Finally, small amounts of DNA are required for this technique (Escaravage et al. 1998), which is particularly advantageous in rare and endangered species. AFLPs are a simple, quick, and cost effective method of estimating genetic diversity (Mace 1999; Muluvi et al. 1999) that can be carried out with standard laboratory equipment.

The disadvantages associated with a particular molecular technique must also be considered in project design. AFLPs are scored dominantly; therefore codominant markers are ignored (Koopman et al. 2001; Nybom 2004). In addition, AFLPs provide fewer polymorphisms when compared to other marker systems (Powell et al. 1996). Finally, this technique targets unknown regions of DNA and the results may represent more than one fragment of equal size from different regions of the genome (Vos et al. 1995).

At present, little research on genetic diversity has been conducted in the Commelinaceae from the molecular or the phenotypic viewpoint. In fact, the only other genetic diversity study in the Commelinaceae is an allozyme study measuring genetic diversity in *Tradescantia hirsuticaulis* (Godt and Hamrick 1993). Allozyme studies have been widely used to study genetic diversity in plants because they are reproducible (Jenczewski et al. 1999), relatively fast and inexpensive (Liu and Furnier 1993) neutral markers (Cruzan 1998). The main disadvantages of this technique are that is does not produce high enough levels of polymorphisms to identify a large number of genotypes present in a population (Cruzan 1998) because the requirement for enzyme activity

limits the range of loci that can be studied (Beebee and Rowe 2004). DNA based techniques often replace or are used in conjunction with allozymes to generate more polymorphisms.

Nowadays, AFLPs are widely used to study intra- and interpopulation genetic variation of endangered and threatened species because of their many advantages (Muluvi et al. 1999; Ribeiro et al. 2002). Genetic variation obtained from AFLP analysis has been used to determine population structure in endangered members of the Fabaceae (Travis et al. 1996), Apiaceae (Gaudeul et al. 2000), and Ericaceae (Escaravage et al. 1998). AFLPs have also been used to study economically important genera of the Rubiaceae (Russell et al. 1999) and Theaceae (Paul et al. 1997). Based on the versatility and advantages listed above, and their ability to detect levels of genetic variation within and among populations in other studies of endangered species worldwide, AFLPs have been chosen to study genetic diversity in *Tradescantia occidentalis*.

### 2.4.2 Statistical Methods to Estimate Genetic Diversity

Several statistical methods have been proposed for the analysis of different types of molecular data, some of which will be applied to this project. In general, the use of statistical methods involves some assumptions about the data set. For instance, the following points are assumed in the estimation of genetic diversity using AFLP data: 1) point mutations at selective bases cause absence of bands, 2) each peak corresponds to only one locus, 3) Mendelian inheritance is in effect, and 4) each locus has two allelic states, resulting in the presence or absence of a band (Gaudeul et al. 2000).

Genetic diversity can be assessed at the intra- and interpopulation levels (Nybom 2004). Among the methods to investigate intrapopulation genetic diversity, Nei's expected heterozygosity, which is the probability that two random alleles in a population can be distinguished with the genetic marker used (Nei and Li 1979), is widely accepted (Muluvi et al. 1999; Gaudeul et al. 2000). Other estimates of intrapopulation variation are Dice's similarity coefficient, Shannon's index of phenotypic diversity and the percentage of polymorphic bands. Dice's coefficient is a pairwise comparison of the data and is similar to Nei's heterozygosity. Shannon's index quantifies and partitions genetic diversity in small sample sizes, and does not require estimates of heterozygosity (Bussell 1999).

Genetic divergence may be assessed at the interpopulation level using Nei's $G_{ST,}$ which is the proportion of diversity calculated from the total genetic diversity in all populations (Culley et al. 2002). $G_{ST}$ values range from zero to one, with low values indicating little variation among populations (Culley et al. 2002). Another method is the F-statistic ($F_{ST}$), which is based on the presence or absence of bands and is operated under the assumption of random mating (Gaudeul et al. 2000). F-statistics involve three parameters that are not affected by sampling methods, size, number of alleles per locus or number of populations sampled, including the associations of: 1) genes within individuals, 2) genes of different individuals at the intrapopulation level, and 3) genes within individuals within a population (Weir and Cockerham 1984). $F_{ST}$ is used to estimate interpopulation diversity (Gaudeul et al. 2000) and population structure (Weir and Cockerham 1984) of polymorphic systems (Excoffier et al. 1992). The values for $F_{ST}$ also range from zero to one, with higher values indicating greater genetic

differentiation among populations (Cardoso et al. 1998). Finally, the Mantel test may be used to investigate correlations between the genetic diversity matrix (F-statistic or G-statistic) and spatial distance (Camacho and Liston 2001). In addition, several statistical packages perform the analysis of molecular variance (AMOVA) (Excoffier et al. 1992), which are used to perform a hierarchical analysis of genetic distance (Muluvi et al. 1999). AMOVA incorporates information regarding DNA haplotype divergence into analysis with fewer assumptions about the statistical properties of the data, making AMOVA a popular program in the biological sciences (Excoffier et al. 1992).

In this study, Dice's coefficient will be used to assess the intrapopulation diversity and Nei's coefficient of genetic distance will be calculated to evaluate interpopulation differentiation in *Tradescantia occidentalis*. Both of these statistics have been applied to AFLP data in other plant species and will allow comparison of the data collected on *T. occidentalis* to other species at risk. In addition, Nei's genetic distance has been used in the genetic diversity study in *T. hirsuticaulis* (Godt and Hamrick 1993).

This study primarily focuses on estimating population size and genetic diversity of *Tradescantia occidentalis*. The incorporation of molecular tools to investigate the levels of genetic diversity in *T. occidentalis* will be particularly useful to implement future conservation strategies. This research will provide insight into the breeding system and degree of reproductive isolation of the Canadian populations of this species as well as valuable ecological information that can serve as a model study for other species at risk in Canada.

## 3. RESEARCH OBJECTIVES

To date, little is known about the ecological requirements and genetic structure of the five Canadian populations of *Tradescantia occidentalis*, and in general the evolutionary history of this and other species at risk  This study was undertaken in response to the lack of understanding of the genetic diversity and general biology of *T. occidentalis*, a threatened species in Canada.  The project was designed to investigate the levels of intra- and interpopulation genetic diversity, as well as to document current population sizes.  It involves a combination of ecological and molecular methods of investigation to address the following main objectives:

1. To conduct a population inventory.

2. To screen levels of genetic diversity at the intra- and interpopulation level of Canadian populations of *T. occidentalis*.

3. To provide guidelines and recommendations for the effective conservation of *T. occidentalis*.

## 4. A POPULATION STUDY IN *TRADESCANTIA OCCIDENTALIS*

### 4.1 Introduction

*Tradescantia occidentalis*, a perennial monocot native to temperate regions of Canada and the United States of America, is nationally threatened (COSEWIC 2005*a*). Considering that a population is defined as a group of individuals separated by at least one kilometre of unsuitable habitat acting as a barrier to propagule distribution (Lancaster 2000), there are five populations of *Tradescantia occidentalis* in Canada (Fig. 4.1). The largest Canadian population of this species is found in the Elbow Sand Hills in south-central Saskatchewan. It is divided into northern and southern polygons on Prairie Farm Rehabilitation Association (PFRA) land and two smaller polygons in the Douglas Provincial Park, henceforth referred to as PFRA (north and south), Douglas, and Highway 19, respectively. Because these areas are separated by less than one kilometer of suitable habitat, they are considered to be a single population. Three additional populations are located in the southwestern corner of Manitoba; one in the Routledge Sand Hills and two in the Lauder Sand Hills. Two of these populations occur on private land, while the third is situated on property owned by the Manitoba Habitat Heritage Corporation (MHHC). The Manitoba populations are henceforth referred to as Routledge, Lauder, and MHHC. The fifth and westernmost population is found on leased and deeded land (Peters 2003*a*) in the dry mixed grass subregion of southeastern Alberta (ANHIC 2004).


**Figure 4.1.** Map of North America showing the distribution of *Tradescantia occidentalis* in Canada and the United States. 1. Alberta population. 2. Saskatchewan population. 3. Routledge (Manitoba) population. 4. MHHC (Manitoba) population. 5. Lauder (Manitoba) population. (modified from Smith 2001).

Although *Tradescantia occidentalis* grows in different habitats across the prairie provinces, it is generally limited to partially stabilized sand dunes or sandy prairies. This species occurs primarily on southwest facing semi-stabilized slopes or active dunes (Godwin and Thorpe 2004) in a rolling sand dune complex in the PFRA subset of the Saskatchewan population and in sandy prairie in the Douglas and Highway 19 polygons. Alternatively, in Manitoba, *T. occidentalis* is found on discrete sand hill formations

(MCDC 2005) and occasionally in small shaded meadows or among low growing shrubs at the base of linear or parabolic sand dunes (Goulet and Kenkel 1997). In Alberta, a few individuals are found on small dunes, but the vast majority of the population grows in gently undulating sandy prairie.

At present, relatively little is known about the ecology, habitat requirements, and associated plant communities of *Tradescantia occidentalis* in Canada, thus these aspects require further study. Furthermore, the effects of grazing and invasive plant species on *T. occidentalis* remain uninvestigated. In addition, an annual assessment of *T. occidentalis* population size is required because of the threatened status of this species. In order to address the above issues, this study has been designed to contribute to the ecological and biological knowledge of this species. The objectives are: 1) to conduct a population inventory to update the estimates of Canadian populations of *T. occidentalis*, and 2) to determine the taxonomic composition of the plant communities within the specified habitat.

## 4.2 Materials and Methods

Field-based population estimates were conducted in each province during the summer of 2005, during which time information on population size and general characteristics (grazed, flowering, and pink-flowered individuals) and associated plant species was collected. Due to differences in landscape features, survey methodologies varied by province as indicated below. Prior to conducting the surveys, it was necessary to establish the definition of a *Tradescantia occidentalis* individual because of disagreement in the use of this term among previous studies. For example, PFRA

researchers consider this species to be rhizomatous; therefore only stems greater than 20 cm apart have been enumerated in their studies. The fleshy fibrous root system of *T. occidentalis* lacks rhizomes (Fig. 2.1B) (K. Remarchuk, pers. obs.; Faden 2000). In some cases, horizontal root growth may be mistaken for rhizomes, but *T. occidentalis* lacks the associated scales and adventitious roots. Consequently, in this study an individual is considered as a multi-stemmed shoot with all stems originating from the same point above ground.

### 4.2.1 Population Estimate

The average density of stems or plants in each population was calculated as the mean number of stems or plants per square metre in each counted polygon or transect. In this study, a polygon is defined as an area that is delineated by GPS coordinates and used to determine the area of occupancy, which is in turn used in the calculation of the average density of this plant. A one-way analysis of variance (ANOVA) was used to determine the variance among the densities and stem to individual ratios in the five populations.

Prior to the study, permission to remove voucher specimens for the Herbarium of the University of Saskatchewan (SASK) and leaf tissue from up to 100 individuals was requested from Saskatchewan Environment and Resource Management (SERM), the Wildlife and Ecosystem Protection Branch of Manitoba Conservation, and Alberta Sustainable Resource Development (ASRD) for the Saskatchewan, Manitoba, and Alberta populations, respectively, to comply with environmental laws and botanical ethics. Permission was granted to remove tissue from only 30 individuals in each

population. In Saskatchewan, the PFRA and Douglas Provincial Park granted permission to access, monitor, and collect plant tissue. In Manitoba and Alberta, landowners and leaseholders were contacted prior to the field season by the Manitoba Conservation Data Center (MCDC) and ASRD, respectively.

#### 4.2.1.1 Population Estimate in Saskatchewan

The Saskatchewan population was surveyed at the end of June, 2005. A collaboration to determine the population number and total number of flowering and grazed plants was established with PFRA researchers to study the large subset of the Saskatchewan population. Their sampling strategy involved constructing 400 m x 3 m transects perpendicular to the dominant landscape features. Nine sites were randomly chosen in the northern polygon and four in the southern polygon (Godwin and Sumners, unpubl.). The average plant density estimated from these transects was applied to the area delineating the polygons to evaluate the total population. Due to the small size of the Douglas and Highway 19 polygons, the total number of stems and individuals in these polygons was counted. The distribution of *Tradescantia occidentalis* on the landscape is seen in Figure 4.2. Global Positioning System (GPS) coordinates were recorded around the perimeter of these polygons using a Garmin eTrex GPS unit (accuracy <3 m).

#### 4.2.1.2 Population Estimates in Manitoba

These population estimates were conducted from July 4-9, 2005. In general, fieldwork was carried out between 5 AM and 1 PM while the flowers were open;

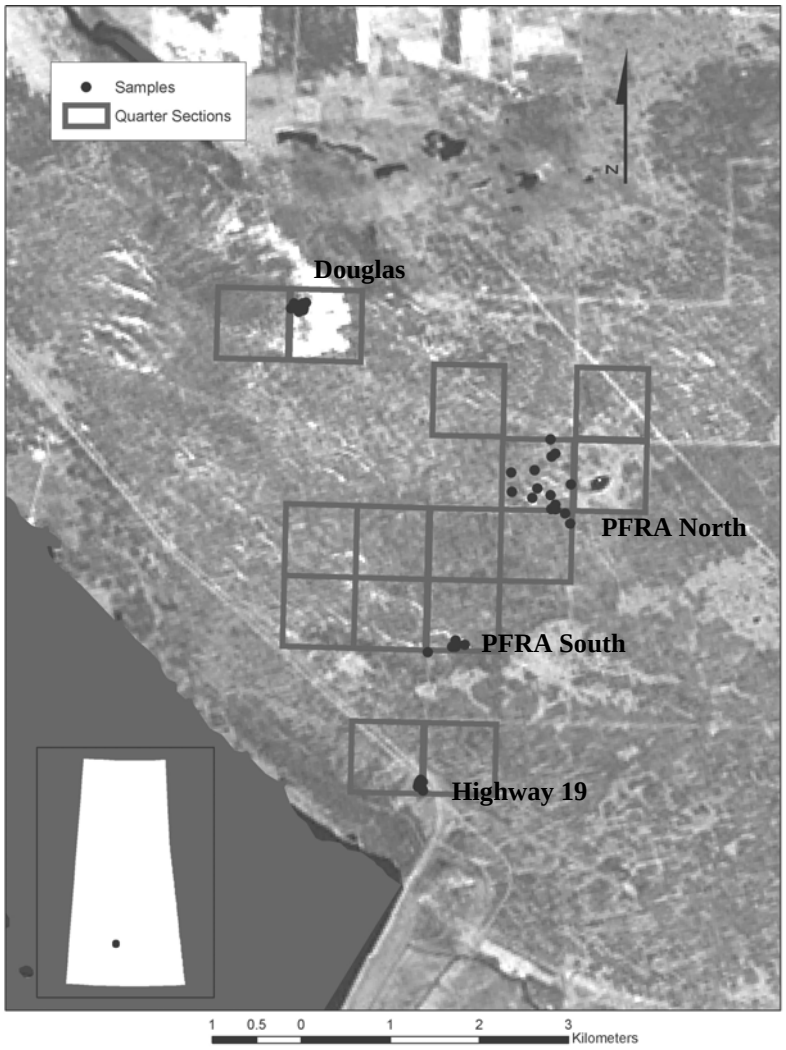


**Figure 4.2.** Landsat image showing the location of the *Tradescantia occidentalis* population in Saskatchewan. Solid gray lines represent quarter sections; light gray and white areas represent exposed sand of partially stabilized sand dunes.

however, due to time constraints, some transect counts were done in the late afternoon. A transect method was chosen to maintain consistency among the Manitoba and Saskatchewan populations but the dimensions differed due of the nature of the sand hill formations. In Manitoba, a 30 m x 6 m wide transect was selected to cover all suitable habitat. In areas where a width of only 15 to 20 m of suitable habitat was present, transects expanded into wooded areas. Transects started at the base of the slope, usually

at the tree line, and were constructed perpendicular to the dune. Numbers between zero and the length of the dune were chosen with a random number generator. Transects were placed "x" metres north of the southernmost point of the dune and recorded with a GPS unit to facilitate the future reassessment of each population.

*Tradescantia occidentalis* was counted in 38 transects in Manitoba. The two ridges in the Routledge population span approximately 3.2 km. Plants were counted in 12 transects on each ridge, for a total of 24 transects. Four transects were enumerated on the 300 m long dune of the MHHC population, while plants in ten transects were counted on the 1.3 km long ridge in the Lauder population. The distribution of plants within each population is illustrated in Figure 4.3. To estimate the total population by extrapolation, the average density in each population was applied to the area of available suitable habitat on each sand hill formation.

#### 4.2.1.3 Population Estimate in Alberta

The Alberta survey was conducted from July 14-18, 2005 as a project funded by ASRD. At the request of ASRD, the methodology was based on previous studies (Peters 2003*a*). This resulted in consistency in the data collected and in an inventory method that suited the local distribution pattern of *Tradescantia occidentalis*. Population estimates were obtained by counting the number of individuals within a polygon, delineating the area with a GPS unit, and calculating average density to be applied to the area known to contain *T. occidentalis* (Remarchuk 2005) shown in Figure 4.4. The number of individuals in each polygon could not be enumerated because of the

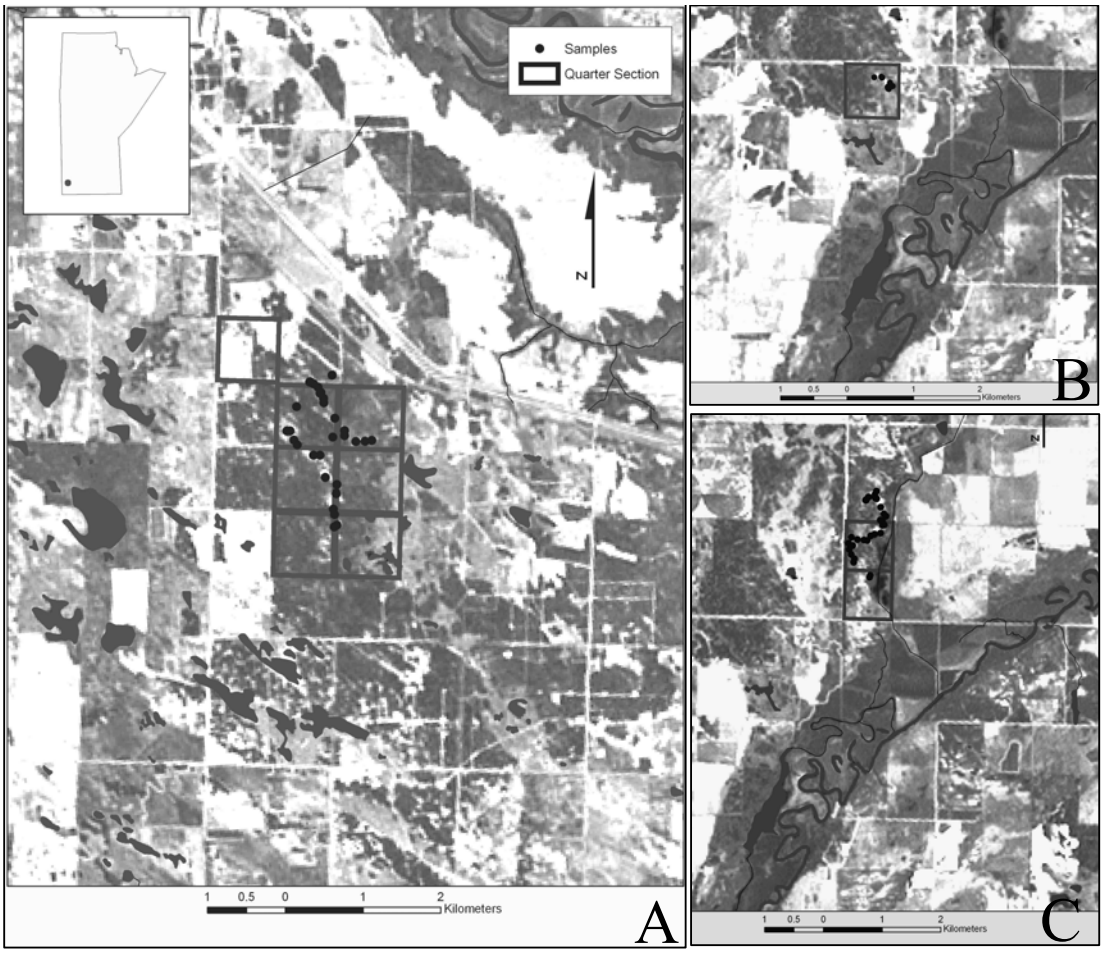


**Figure 4.3.** Landsat images showing the location of the *Tradescantia occidentalis* populations in Manitoba. **A.** The Routledge population. **B.** The MHHC population. **C.** The Lauder population. Solid gray lines represent quarter sections; light gray and white areas represent exposed sand or more commonly, crops.

large population size and time constraints. The average density was extrapolated to the area calculated using ArcView 3.2 (ESRI) of the uncounted polygons. The total population estimate was obtained by adding the counted and extrapolated polygons. Searches for additional patches of this species were conducted by walking through the study area, which was inclusive of, but not limited to, the sand dune complex.

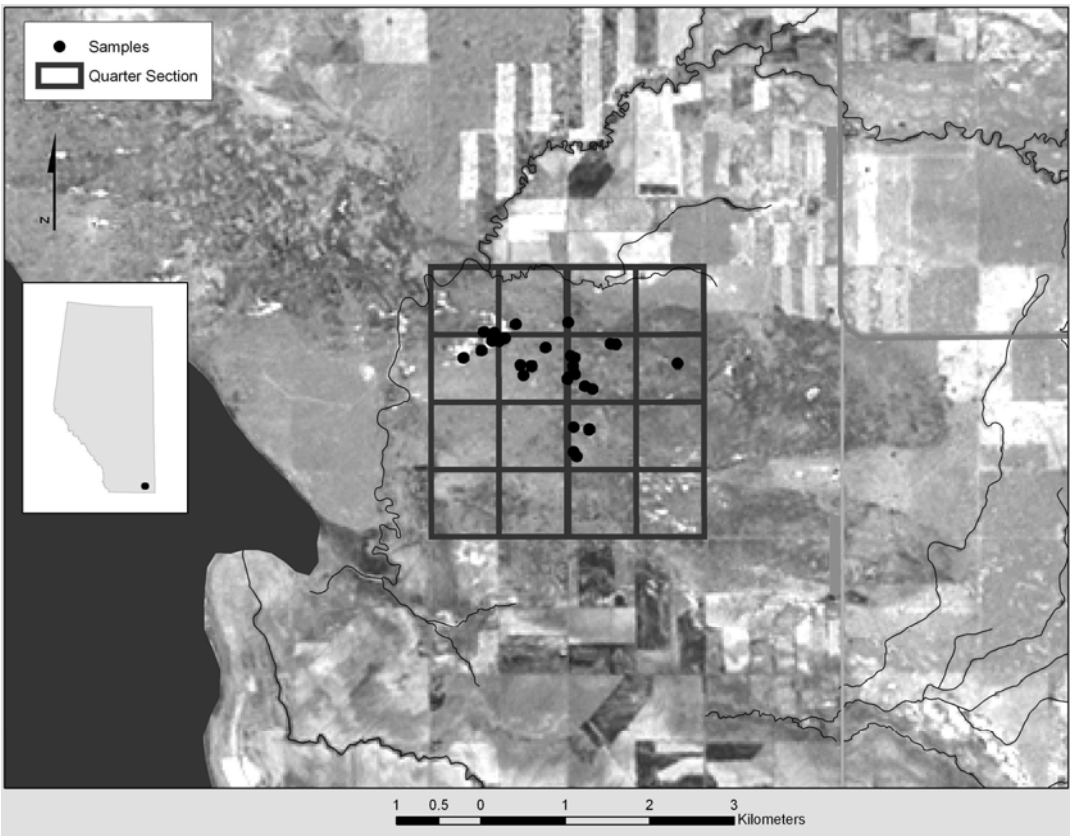


**Figure 4.4.** Landsat image showing the *Tradescantia occidentalis* population in Alberta. Solid gray lines represent quarter sections; light gray and white areas represent exposed sand or crops.

### 4.2.2 Associated Species

In order to characterize the taxonomic composition of the community types, species found in areas with and without *Tradescantia occidentalis* were recorded. For ease of reference, areas with *T. occidentalis* are henceforth referred to as Habitat Type A and areas without *T. occidentalis* as Habitat Type B. A 1 $m^2$ plot was established every 5 m along 25 m transects in Habitat Type A to document the plant species. In Saskatchewan, five transect locations were chosen using a random number generator within the coordinates delineating the population (Godwin and Thorpe 2004). Species in selected polygons or transects in Habitat Type A were recorded in a total of 18 transects in Manitoba and seven in Alberta. Locations in Habitat Type B were targeted

within the study area for comparative purposes between the two habitat types. For Habitat Type B, species in five transects were recorded in Saskatchewan and Manitoba, and two in Alberta. Using the statistical program PC-ORD v.4 (McCune and Mefford 1999; McCune and Grace 2002), a correspondence analysis (CA), also known as reciprocal averaging (RA) analysis, was performed on the combined transects and separately on transects in Habitat Types A and B. Downweighting of rare species was selected for all analyses.

## 4.3 Results

### 4.3.1 Population Estimates

Due to the sensitivity of rare plant location information, polygon and transect coordinates are not provided here but are available through SERM, MCDC, or Alberta Natural Heritage Information Centre (ANHIC) for Saskatchewan, Manitoba, and Alberta, respectively.

With the exception of the PFRA polygons, the density values given below are in terms of stems instead of plants because of the consistent stem definition in the literature. The density values of flowering, grazed, and pink-flowered plants are provided in Appendix I and represented graphically (Fig. 4.5) but are not reported in the results because only general trends are discussed. The raw and calculated data for stems and plants collected in each province is provided in Appendix I, including data for: Saskatchewan (PFRA) (Table AI.1), Saskatchewan (Highway 19 and Douglas) (Table AI.2), Routledge (Table AI.3), MHHC (Table AI.4), Lauder (Table AI.5), and Alberta

(Table AI.6). A comparison of the population data from 2005 with previous estimates is found in Table AI.7 of Appendix I.

#### 4.3.1.1 Population Estimate in Saskatchewan

The raw data presented here for the PFRA polygons is based on personal communication with PFRA researchers. The north polygon covers approximately 485,770 $m^2$, where a total of 1,269 plants were counted in nine transects. This subset of the population is estimated at 56,359 ± 38,635 individuals (95% confidence level) with an average density in the north polygon of 0.1175 plants/$m^2$. Approximately 63% of plants were flowering and 47% showed evidence of grazing at the time of the survey. In the south polygon, which covers an area of approximately 107,666 $m^2$, 59 plants were observed in four transects, yielding a population estimate of 1,340 ± 4,261 individuals (95% confidence level). The average density in this polygon was 0.0491 plants/$m^2$ (Table AI.1).

The Douglas and Highway 19 polygons of the Saskatchewan population encompassed areas of 11,887 $m^2$ and 5,895 $m^2$, respectively. A total of 696 stems (430 plants) in Douglas and 481 stems (260 plants) in Highway 19 were counted (Table AI.2). The average stem density of these two polygons is 0.0700 stems/$m^2$. The mean proportion of flowering and grazed stems is 81.6 % and 3.3%, respectively. Searches in suitable habitat adjacent to the known polygons of *Tradescantia occidentalis* were unsuccessful in the discovery of additional individuals. The density and proportion of flowering, grazed, and pink-flowered stems from PFRA north and south, Douglas and Highway 19 have been combined for analytical purposes (Figs. 4.5 and 4.6).

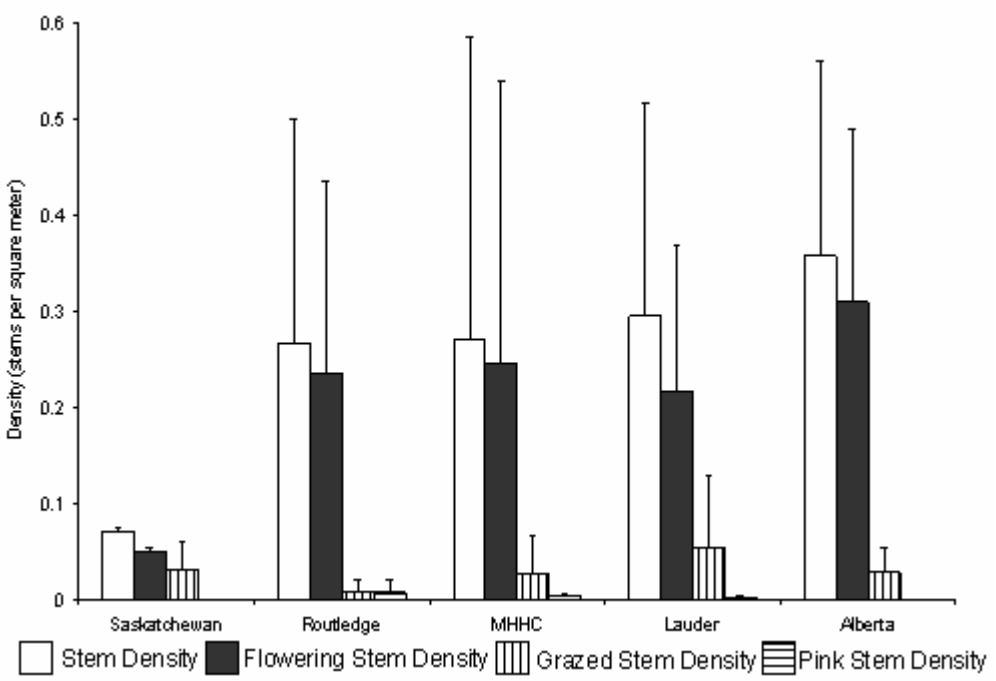


**Figure 4.5.** Graphic representation of the average total, flowering, grazed and pink-flowered stem density in the five Canadian populations of *Tradescantia occidentalis*. Bars represent standard deviation.

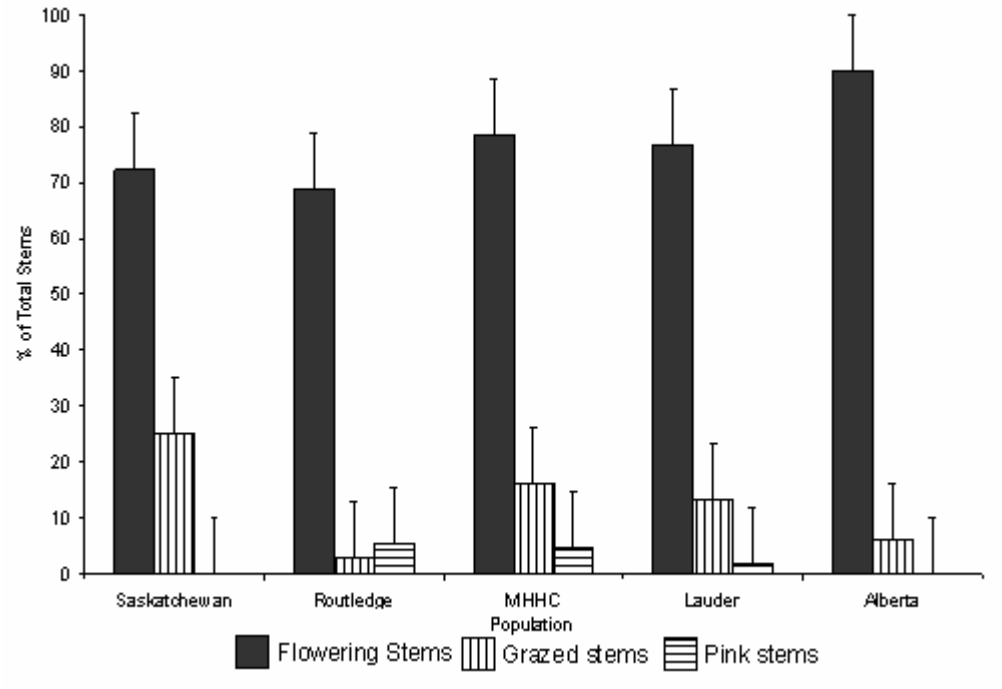


**Figure 4.6.** Graphic representation of the proportion of flowering, grazed and pink-flowered stems in the five Canadian populations of *Tradescantia occidentalis*. Bars represent standard deviation.

#### 4.3.1.2 Population Estimates in Manitoba

In this 2005 survey, a total of 1,148 stems (701 plants) were counted along 24 transects in the Routledge population, which is estimated at 21,948 ± 8,344 stems (13,402 ± 4,170 plants) with a 95% confidence level (Table AI.3). The average density is 0.2657 stems/$m^2$ (Fig. 4.5). An estimated 68.7 % stems have reproductive structures, 3.0% are browsed, and 5.5% have pink flowers (Table AI.3; Fig. 4.6).

In the smallest Canadian population of *Tradescantia occidentalis*, located on MHHC property, a total of 194 stems (62 plants) were counted in four transects (Table A1.4). The total population is estimated at 2,425 ± 7,261 stems (775 ± 3,511 individuals) with a 95% confidence level (Table A1.4) from an average density of 0.2694 stems/$m^2$ (Fig. 4.5). An estimated 78.6% of stems have reproductive structures and 16.2% are browsed, while approximately 4.7% bear pink flowers (Table AI.4; Fig. 4.6).

In the Lauder population, 530 stems (179 plants) were counted in ten transects. This population is estimated at 11,916 ± 6,796 stems (4,024 ± 1,530 individuals) with 95% confidence (Table A1.5). The average density is 0.2944 stems/$m^2$ (Fig. 4.5) and an estimated 76.8% of stems have reproductive structures, 13.2% are grazed, and 1.9% are pink flowered (Table AI.5; Fig. 4.6).

#### 4.3.1.3 Population Estimate in Alberta

As previously mentioned, the Alberta population survey was funded by ASRD and the population numbers reported here are the outcome of that inventory (Remarchuk 2005). A total of 3,627 stems (1,861 plants) were counted in 11 polygons during the

2005 field season. The total population was estimated at 56,834 ± 19,188 stems (28,430 ± 11,682 individuals) with a 95% confidence level (Table AI.6). The average density is 0.3254 stems/$m^2$ (Fig. 4.5). An estimated 90% of stems have reproductive structures, while only 6.1% are browsed (Fig. 4.6).

### 4.3.2 Parameter Comparison Among Populations

In general, the Alberta population of *Tradescantia occidentalis* has the highest total (0.3254 stems/$m^2$) and flowering stem (0.3088 stems/$m^2$) densities. In turn, the Saskatchewan population has the lowest estimates in nearly all categories, while the density values among the Manitoba populations are relatively similar (Fig. 4.5). The proportion of flowering stems is comparable in the five populations; however, the highest proportion of grazed stems occurs in Saskatchewan (81.6%) (Fig. 4.6). The highest proportion of pink-flowered stems is observed in Routledge (5.5%) (Fig. 4.6). For exact values of parameters estimated in Figs. 4.5 and 4.6, refer to Appendix I.

One-way ANOVAs were conducted on stem densities calculated in the five populations to infer the relationships among the parameters investigated. For all parameters, the null hypothesis is that the densities are the same among the populations. The null hypothesis is rejected for grazed stem density, which indicates that grazing differs among the populations. This is expected due to the implementation of different management strategies. For all other parameters the null hypothesis cannot be rejected (Table 4.1) because the calculated F value is less than the critical F value obtained from statistical tables (Campbell 1989). The probability values are high ($p>0.1$), indicating

that the decision to fail to reject the null hypothesis is not strongly supported (Table 4.1).

**Table 4.1.** Statistical summary of the one-way ANOVA for plant and stem densities at five populations of *Tradescantia occidentalis* in Canada.

| | **Stem Density** | **Individual Density** | **Flowering Stem Density** | **Flowering Individual Density** | **Grazed Stem Density** | **Grazed Individual Density** | **Pink Density (MB only)** |
|---|---|---|---|---|---|---|---|
| Variance among populations | 0.0400 | 0.0166 | 0.0329 | 0.0201 | 0.0042 | 0.0005 | 0.0001 |
| Variance within populations | 0.0579 | 0.0107 | 0.0411 | 0.0109 | 0.0015 | 0.0014 | 0.0001 |
| F value | 0.6901 | 1.5404 | 0.8022 | 1.8512 | 2.7049 | 0.3707 | 1.2053 |
| p value | 0.60 | 0.21 | 0.53 | 0.14 | 0.04 | 0.83 | 0.31 |
| *Accept or reject null hypothesis** | Fail to Reject | Fail to Reject | Fail to Reject | Fail to Reject | Reject | Fail to Reject | Fail to Reject |

***** The F value with df(4,40) is 2.61, at p=0.05

### 4.3.3 Associated Plant Species

Several plant species associated with *Tradescantia occidentalis* populations are common across the prairie provinces. Plots of RA analysis show clustering of transects on two axes based on the similarity and abundance of the plant species documented, which theoretically should reflect species composition in each habitat type and province. The variables represented by axes one and two are unknown. Relatively little of the variance seen on two axes is explained in the RA analysis (Table 4.2), with the highest proportion of the variance explained in Habitat Type B (Table 4.2). The proportion of the distance explained, which is a measure of how well the distances in the ordination represent the data in the original matrix, is also low (Table 4.2).

**Table 4.2.** Summary of RA analysis with downweighting selected for Habitat Type A, Habitat Type B, and combined analysis (Habitat Types A and B). The variance is the amount of variation in the original data matrix that can be partitioned between the three provinces.

| | **Habitat A** | **Habitat B** | **Habitats A and B** |
|---|---|---|---|
| **# Non-zero items** | 576 | 210 | 651 |
| **Total variance (inertia)** | 2.2179 | 1.3172 | 3.1231 |
| **Variance on Axis 1 (%)** | 15.3 | 33.0 | 11.0 |
| **Variance on Axis 2 (%)** | 11.8 | 17.8 | 10.6 |
| **Total Variance (%)** | 27.1 | 50.8 | 21.6 |
| **Total Distance** | 0.296 | 0.673 | 0.441 |
| **Distance on Axis 1 (%)** | 9.4 | 13.5 | 3.6 |
| **Distance on Axis 2 (%)** | 20.9 | 46.0 | 33.4 |
| **Total % Distance** | 29.6 | 59.5 | 37.0 |

The community types in the three provinces differ slightly, as seen in the combined RA analysis (Fig. 4.7) because of the abundance of some taxa in each population. The RA analysis of Habitat Type B also shows a differentiation in habitat type among the provinces (Fig. 4.8) based on the presence of the following species: *Thermopsis rhombifolia*, *Koeleria macrantha*, *Populus tremuloides*, *Arctostaphylos uva-ursi*, *Selaginella densa*, *Prunus virginiana*, *Opuntia fragilis*, *O. polyacantha*, *Artemisia campestris* (Saskatchewan), *Andropogon hallii*, *Cyperus schweinitzii* (Manitoba), *Artemisia ludoviciana*, and *A. frigida* (Alberta). However, Habitat Type A shows less distinction in plant communities (Fig. 4.9), which are influenced by *Galium boreale*, *Vicia americana*, *Maianthemum stellatum*, and *Symphoricarpos occidentalis*. Based on an inventory of the plant species in Habitats A and B, no major floristic differences were found among the populations. Several common species not associated with *Tradescantia occidentalis* in one province, such as *Asclepias viridiflora* and *Heterotheca villosa*, were found in association with *T. occidentalis* in other provinces (Appendices II and III). In general, Habitat Type A is taxonomically richer than Habitat Type B in each province (Fig. 4.10), and as expected, the compilation of the floristic

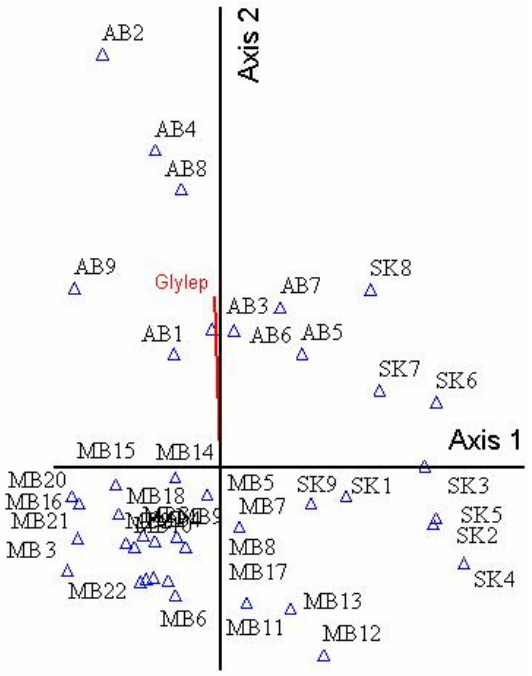


**Figure 4.7.** Binary plot of the RA analysis of transects evaluated in Habitat A and Habitat B. The cutoff value for species is 0.50.

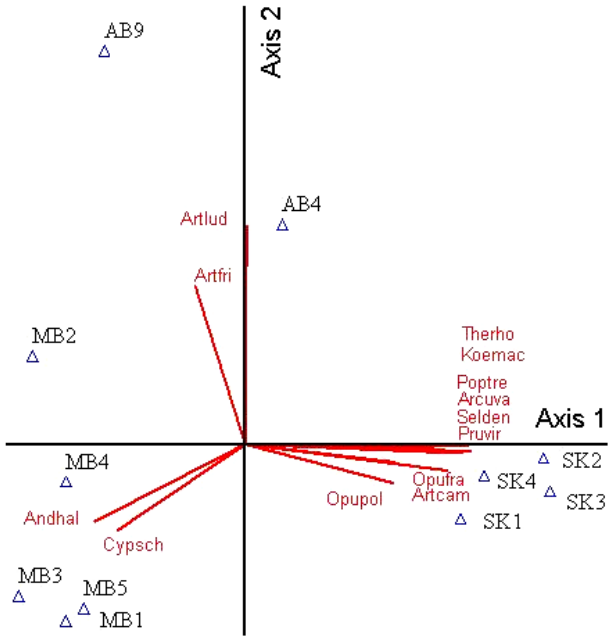


**Figure 4.8.** Binary plot of the RA analysis of transects evaluated in Habitat B. The cutoff value for species is 0.50.

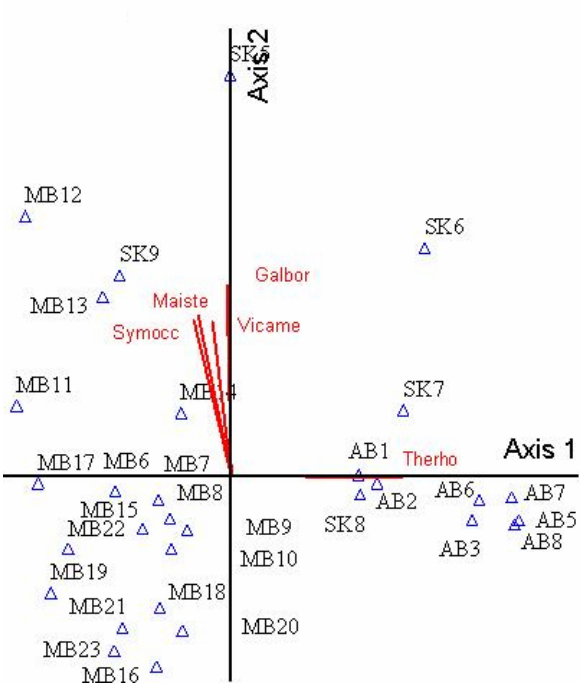


**Figure 4.9.** Binary plot of the RA analysis of transects in Habitat A. The cutoff value for species is 0.50.

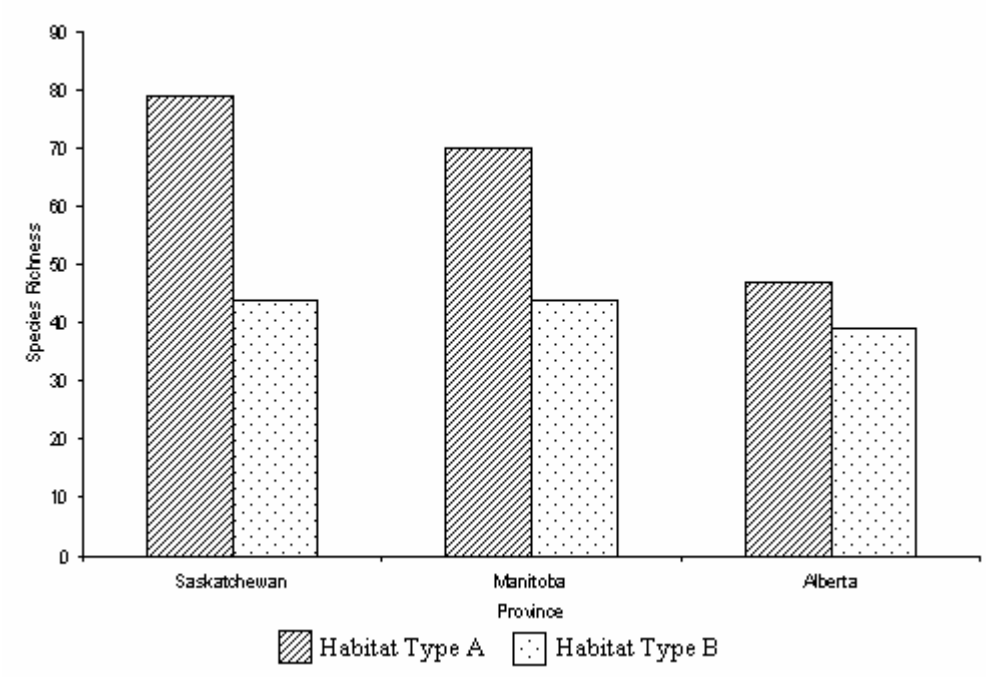


**Figure 4.10.** Graphic representation of species richness in Habitat Type A and Habitat Type B in the Canadian prairie provinces.

elements in the two habitats surpasses that of Habitat Type A with a total of 123 species (93 genera in 35 families) (Table 4.3). A breakdown of the number of families, genera, and species by province is provided in Table 4.3.

**Table 4.3.** Number of families, genera, and species in Habitat Types A, B, and Combined Habitats for Saskatchewan, Manitoba, and Alberta.

| | **Habitat Type A** | | | | **Habitat Type B** | | | | **Combined Habitat Types A & B** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **SK** | **MB** | **AB** | **Total** | **SK** | **MB** | **AB** | **Total** | **SK** | **MB** | **AB** | **Total** |
| # Families | 28 | 28 | 19 | 34 | 21 | 21 | 18 | 31 | 28 | 29 | 19 | 35 |
| # Genera | 62 | 64 | 41 | 86 | 48 | 50 | 45 | 67 | 65 | 66 | 47 | 93 |
| # Species | 80 | 70 | 47 | 114 | 61 | 57 | 52 | 96 | 82 | 73 | 51 | 123 |

Other taxa observed on the PFRA pasture in Saskatchewan include provincially ranked species at risk such as *Cyperus schweinitzii* and *Elymus lanceolatus* subsp. *psammophilus*. In the MHHC population in Manitoba, *Tradescantia occidentalis* was observed growing in association with *Campanula rotundifolia*, a finding that is in disagreement with previous reports (C. Hamel, MCDC, pers. comm.). *Euphorbia esula*, a noxious species, is abundant in the Saskatchewan and Manitoba populations, but is absent in Alberta. A taxonomic list of species recorded during the surveys is provided in Appendices II and III for Habitat Types A and B, respectively.

## 4.4 Discussion

### 4.4.1 Population Estimates

Based on the 2005 estimates, there is a perceived increase in the number of individuals in several populations of *Tradescantia occidentalis* (Table AI.7). Previous studies of this species in Alberta indicate that high levels of precipitation are correlated with increases in population number (Smith 2002; Peters 2003*a*; Peters 2003*b*). In this

study, data collected from the Alberta population supports this theory. However, this observation is not consistent among provinces. The 2005 PFRA inventory in Saskatchewan indicates a relatively stable population number (Godwin and Sumners, unpubl. data; Godwin and Thorpe 2005), suggesting that size is not directly related to precipitation. Slight differences in population size can be expected due to sampling error or environmental variability (Lande 1999), for example, there may be fewer individuals in drier years. The use of different sampling methods among studies may have implications in the accuracy of the estimation of previous and current population size (Table AI.7); therefore, possible correlations between precipitation and population fluctuations should be interpreted with caution.

#### 4.4.1.1 Saskatchewan Population

As indicated earlier in this chapter, population estimates for the Saskatchewan population (PFRA land) were conducted by PFRA researchers in 2005. The Saskatchewan population estimates for the north (56,359 plants) and south (1,340 plants) polygons on the PFRA land in 2005 (Godwin and Sumners, unpubl.) are relatively similar to the 2004 estimates of 56,583 and 4,951 plants reported by Godwin and Thorpe (2005) in the north and south polygons, respectively (Tables AI.1, AI.7). In 2003, the north and south polygons were estimated at 38,376 individuals and 2,907 individuals, in that order (Godwin and Thorpe 2004). The population estimates in 2005 and 2003 are 99% and 68% of the 2004 estimate, respectively (Table AI.7) for PFRA north. In the south polygon, the 2005 and 2003 estimates are 27% and 58% of the 2004 estimate (Table AI.7). According to Godwin and Thorpe (2004), in spite of the

substantial variation in population number, the estimates are not statistically different due to large error margins associated with the sampling method used in this study.

Unlike the PFRA population, the 2005 estimates obtained in this study for the Douglas and Highway 19 polygons are lower compared to the 2004 estimates. In this survey, the Douglas polygon is estimated at 696 stems versus 2,137 in 2004 (SERM 2005), which represents a 67% decrease (Table AI.7). In the Highway 19 subpopulation, the number of stems is also lower and estimated at 481 compared to 758 in 2004 (SERM 2005). The 2005 estimate represents 63% of the individuals of the 2004 estimate (Table AI.7). The 2005 estimates are considerably lower, suggesting that these populations have declined. This decrease may be explained by the fact that fewer individuals were found because the Saskatchewan inventory was conducted earlier in the season and some plants without flowers may have been overlooked.

#### 4.4.1.2 Manitoba Populations

The 2005 estimates of the Manitoba populations are comparable to previous inventories. In this study, the Routledge population is projected to be 13,402 individuals compared to 9,422 individuals in 2001 (MCDC 2005). The 2005 estimate represents 142% of the 2001 survey (Table AI.7). In contrast, the MHHC population has remained stable with an estimated 775 plants in 2005 and 787 individuals in 2001 (MCDC 2005). This represents a 2% decrease from 2001 (Table AI.7). Statistically, the error margin is high (775 ± 3,511 plants) because of the few of transects sampled, but considering the small population size and the proportion of individuals counted, the above calculation is regarded as accurate. On the other hand, the Lauder population shows a minor decline

in number of individuals relative to previous estimates. The population was estimated at 4,024 plants in 2005, which is a 7% decline (Table AI.7) compared to 4,321 plants in 2001 (MCDC 2005). However, this data is in disagreement with the observations of the landowner (pers. comm.) of the Lauder population, who indicated the presence of more *Tradescantia occidentalis* in 2005 than in previous years. This further suggests that the observed decline is in part due to sampling error, but plant phenology and environmental fluctuations should also be taken into consideration.

#### 4.4.1.3 Alberta Population

The Alberta population was originally located in the mid 1980s (Smith 2001), but until 2002 inventories focused solely on areas of the semi-active sandy environments in the dune complexes. In 1990, the population was estimated at 210 individuals (Smith 2002) (1% of 2005 estimate, Table AI.7) but only seven individuals were located in 2001 (Smith 2001). The 2002 survey reported a population of 7,450 individuals (Peters 2003*a*) (26% of 2005, Table AI.7), while the 2003 estimate was 7,700 plants (73% less than 2005, Table AI.7) (Peters 2003*b*). The 2005 estimate of 28,430 plants (Remarchuk 2005) exceeds previous reports by a factor of approximately four.

Several factors may account for the discrepancies among these estimates. Since the majority of the population is found in sandy prairie, it is possible that a large proportion of individuals were missed in early studies. Although the 2002 and 2003 inventories included a wider study area on partially active dunes and the surrounding prairie, several new patches of *Tradescantia occidentalis* were located in this study. It is evident that the perceived population increase in 2005 is due to the discovery of new

patches. In addition, the polygons from this study were larger in size and number than those in previous studies (ANHIC 2005), which is putatively indicative of the wider distribution of this species. Another possible factor is that *T. occidentalis* is difficult to locate among other prairie plant species in its grass-like vegetative stage. However, some plants were beginning to senesce at the time of the Alberta inventory, which facilitated the field identification of this species.

### 4.4.2 Population Density

Total stem density was recorded primarily for the purpose of estimating population size. This value can also be used as an indicator of ideal growing conditions because plants develop and proliferate better in optimum environments. Among the Canadian populations, the Saskatchewan population has the lowest average stem density, while Alberta has the highest (Tables AI.1, AI.2, AI.6; Fig. 4.5). The habitats differ between these two provinces, thus higher density values may indicate that *Tradescantia occidentalis* is better suited to the prairie conditions in Alberta. Higher plant density may also reflect differences in carrying capacities and levels of species competition in the different environments (Lande 1999). It must be noted that resource overlap among species does not measure competition and when resources are not limited, resource use of different species may coincide significantly (Sale 1974). Thus, the higher density values observed in Alberta are not necessarily correlated with lower intra- or interspecific competition rates.

It has been hypothesized that pollinator visitation rates increase in dense populations because pollinator energy intake increases with high flowering density

(Schmitt 1983). However, studies have shown that there is no difference in the pollinator visitation rates per flower among populations with high and low densities in the Ranunculaceae (Bosch and Waser 1999) and Boraginaceae (Klinkhamer and de Jong 1990). Although no direct measurements on the number of pollinators were recorded in the Canadian populations of *Tradescantia occidentalis*, more pollinators were observed in the more dense Alberta population (K. Remarchuk, pers. obs.); however, this could have been due to the presence of more flowers and may not reflect pollinator visitation rates.

In *Tradescantia occidentalis,* pink-flowered stems, which are found in low frequency in Manitoba, occur in very close proximity to blue-flowered stems with no apparent difference in macroenvironment. The Routledge population has the highest proportion of pink flowers, followed by MHHC and Lauder (Fig. 4.5). No difference in the frequency of pollinator visitation was observed for the pink-flowered plants (K. Remarchuk, pers. obs.); however, this parameter was not measured. The specifics of flower colour variation and its relationship to pollinator attraction in *T. occidentalis* are obscure and the genetic basis of flower colour polymorphism in this species requires further investigation.

### 4.4.3 Associated Species

Although plant species associated with *Tradescantia occidentalis* have been previously recorded, no community analysis has been completed prior to this study. An analysis of the plant communities that occur in *T. occidentalis* habitat was conducted to determine if this plant is associated with particular species, for which more of the

ecological requirements are known. The separation of provinces along each axis of the RA analyses is difficult to explain in terms of environment, though there are several hypotheses. Transects in Habitat Types A and B were very similar except that Habitat Type B lacked *T. occidentalis*; therefore, the following ideas can be applied to both habitat types. One possible explanation is the nature of the dune formations. The Manitoba populations of *T. occidentalis* occur on discrete dunes whereas in Saskatchewan and Alberta, plants are dispersed throughout an area of suitable habitat. In addition, the successional stage of the dunes varies in each province. The most active dune complexes occur in Saskatchewan and Manitoba and the most stabilized dunes are found in Alberta. Also, slope size may be influential because *T. occidentalis* occurs on large, moderate, and gently rolling dunes in Saskatchewan, Manitoba, and Alberta, respectively. The individual or combined effect of these factors may contribute to the clustering patterns of transects by province in the RA analysis (Figs. 4.7-4.9).

According to the RA analysis in Habitat Type A (Fig. 4.9), species influencing the separation of the Manitoba transects are *Maianthemum stellatum* and *Symphoricarpos occidentalis*. Interestingly, these species are infrequent on dunes in Manitoba but are common in Saskatchewan or Alberta. This indicates the need for more transects in Habitat Type A because theoretically, species influencing the separation of populations at the provincial level should be locally common or unique to that province. Another species, *Thermopsis rhombifolia*, influences the separation of the Saskatchewan transects because it is associated with stabilized areas at the edges of *Populus tremuloides* forest in the study area where occasional individuals of *Tradescantia occidentalis* are found. In turn, in Habitat Type B, *Artemisia ludoviciana* and *A. frigida*

influence the clustering of the Alberta transects, *Andropogon hallii* and *Cyperus schweinitzii* influence the distinction of Manitoba, and species such as *T. rhombifolia*, *Selaginella densa*, and *Opuntia fragilis* influence Saskatchewan (Fig. 4.8). These results are more realistic because some of these species are common in their respective provinces but are not frequently observed elsewhere. In addition, *A. hallii* is native to Manitoba but is not known to occur naturally in Saskatchewan or Alberta.

Taxonomically, the species diversity in Habitats A and B is not significantly different but in general, the former has a higher number of species than the latter. Some taxa, such as *Toxicodendron radicans* in Saskatchewan, were not recorded outside the Habitat A transects, but were evident on the landscape. Furthermore, some species do not occur in certain provinces, such as *Asclepias viridiflora* var. *linearis* in Alberta and *Andropogon hallii* in Alberta or Saskatchewan. In Habitat A and the combined analysis, the provinces separate indistinctly, indicating that the communities in which *Tradescantia occidentalis* occurs do not differ because of the association with *T. occidentalis* but because of taxonomic differences in species among provinces which may vary due to the amount of stabilization of the dunes. For example in Alberta, the dunes are much more stabilized than in Manitoba and Saskatchewan and thus plants associated with active sand are not abundant in Alberta. A taxonomic list of plants in Habitat Types A and B is included in Appendices II and III, respectively.

### 4.5 Conservation Management of *Tradescantia occidentalis*

Coordinated research is needed to facilitate preservation of local, national, and global biodiversity. In Saskatchewan, a National Recovery Team for Plants at Risk in

the Prairie Provinces has been established. Its goal is to survey and inventory species at risk, but to date has not formulated an action plan regarding *Tradescantia occidentalis*. However, in early 2005, a maintenance and recovery plan for this species was drafted by the Alberta Western Spiderwort Recovery Team (2004), which includes members of the Alberta government, a conservation agency, and members of the general public. The action plan involves population and habitat conservation and management, public information and education, and research into knowledge gaps. Resource acquisition, legislation and plan management, and administration are also considered.

Conservation management includes annual population surveys with standardized estimation protocols and surveys for invasive species. These protocols should be consistent to increase the accuracy of population fluctuations from year to year. The polygon extrapolation method designed by Peters (2003*a*) and employed in the 2005 survey of *Tradescantia occidentalis* worked well for the spatial distribution of the Alberta population; however, the method is not strongly supported statistically. The statistical support may be strengthened by increasing the number of polygons counted. The same premise holds for the sampling strategies employed in the Manitoba and Saskatchewan populations of *T. occidentalis*. Therefore, it is recommended that consistent sampling methods are used in future surveys and that polygons counted in this study be enumerated in subsequent surveys. Additional patches of *T. occidentalis* may be identified in future studies, thereby changing the overall population estimations. Monitoring changes in numbers by counting known polygons is more valuable to the understanding of this species because it reduces the error due to inconsistent sampling

techniques. This will allow meaningful correlations between environmental conditions, grazing, and other factors in relation to population size.

Though *Tradescantia occidentalis* does not appear to be severely affected by the invasion of *Euphorbia esula* at this time, further investigation into new methods of biological control should be explored as a conservation management strategy. The long term effect of invasive species on native plants is sometimes unpredictable but can be very serious. If *E. esula* is not controlled, the Saskatchewan and Manitoba populations may decline because of dune stabilization and the allelopathic effect of decaying *E. esula* litter (Steenhagen and Zimdahl 1979). At present, there is no method of eradicating this species from the plant community. However, in the MMHC population in Manitoba, methods of biological control, such as *Hyles euphorbia* L. (spurge hawkmoth) and *Aphthona lacertosa* Rosenheim (leafy spurge beetle), have been implemented in an effort to prevent the spread of *E. esula* (MCDC, pers. comm.; Pachkowksi 2002). In addition to biological control, it is recommended that other methods of extirpation of this invasive species, such as grazing by sheep or herbicide application, be investigated. The implementation of new methods should be used with caution as they may be detrimental to *T. occidentalis* and other native plants.

Grazing by native ungulates is another factor affecting *Tradescantia occidentalis* because it removes the reproductive structures. Grazing may lead to the increase in the number of vegetative stems and involves the removal of flowers or capsules with the consequent inability of plants to produce offspring by sexual reproduction. Since *T. occidentalis* does not spread through rhizomes, the production of viable seeds is important to propagate the species. If grazing is early in the season, the stems often

regenerate and produce flowers, which may actually be beneficial to the propagation of this species; however, it was observed that grazing tends to produce many non-reproductive stems that had not flowered at the time of the study (K. Remarchuk, pers. obs.). It should be noted that historically, *T. occidentalis* has been subjected to natural grazing cycles by bison, deer, and possibly moose, and thus is adapted to some level of grazing. Based on the status of the populations, current grazing regimes do not appear to have a negative impact on the populations. However, in the future, overgrazing may be a potential threat to *T. occidentalis* and may also favour the establishment of invasive species, particularly if bales containing seeds of invasive taxa are used as a supplementary food source for cattle.

No requests for oil and gas exploration have been reported for these areas in Saskatchewan and Manitoba. In Alberta, there is currently no petroleum exploration in the area of the *T. occidentalis* population, though this practice has been considered in the past and is likely to continue to be an issue in the future. Despite the relatively stable or increasing population numbers, it is important to consider the threats surrounding the populations of *T. occidentalis* and enforce environmental laws in this and other Canadian species at risk.

### 4.6 Concluding Remarks

Several conclusions regarding the demography, association of *Tradescantia occidentalis* with other plant species including invasive taxa, and effects of grazing in the Canadian populations can be made from this study. In Saskatchewan and Manitoba, the population estimates were relatively similar to the previous survey and in Alberta the

estimated population size in 2005 greatly exceeded previous estimates. The nationally and provincially threatened status of *T. occidentalis* is supported even though the population data from this study suggests that *T. occidentalis* is not critically endangered or in danger of extinction. Generally, demographic stochasticity in populations greater than 100 individuals is of relatively little importance (Lande 1999) and is unlikely to be a problem despite the large discrepancies in population estimates among this study and previous estimates in the Alberta and Saskatchewan populations. The International Union for the Conservation of Nature (IUCN) ranking is based on the number of populations in each province, an area of occupancy less than 20 $km^2$, and an overall extent of occurrence less than 5,000 $km^2$ (IUCN 2005); therefore the large size of each population does not warrant a change in status.

The association of *Tradescantia occidentalis* with other plants within each province revealed that this species is not associated with any particular taxa. The plant communities differ by species composition by province and not by association with *T. occidentalis*. The invasive *Euphorbia esula* was found extensively throughout the Saskatchewan and Manitoba populations but *T. occidentalis* appears to be capable of co-existing with this species at present. Further research and monitoring of *E. esula* is recommended. Grazing in all Canadian populations is limited and does not appear to be detrimental to the survival of *T. occidentalis* in Canada. However, upon further fragmentation of the habitat, increases in density of *E. esula*, or increased grazing pressure, current populations of this species could rapidly decline. In brief, *T. occidentalis* should remain protected because of the small number of Canadian populations and their reproductive isolation. The protection of *T. occidentalis* will also

prevent further fragmentation of the delicate sand dune environment and other species at risk. Since various fundamental aspects regarding the biology and natural history of this species require further investigation, it is recommended that subsequent studies focus on key issues outlined in this study, including response to grazing and invasive plant species.

# 5. A STUDY OF GENETIC DIVERSITY IN CANADIAN POPULATIONS OF *TRADESCANTIA OCCIDENTALIS*

## 5.1 Introduction

*Tradescantia occidentalis* (Commelinaceae) is a perennial monocot native to Canada and the United States of America. In areas of the United States larger populations of this species are found in a continuous distribution pattern; however, only five populations exist in Canada (Fig. 5.1). The distribution has been discussed in Chapter 4, but it is important to briefly revisit this topic to remind the reader of the locations of the Canadian populations. The northernmost population is located in the Elbow Sand Hills of central Saskatchewan (Fig. 5.1). Three populations are situated in southwest Manitoba, two in the Lauder Sand Hills (MHHC and Lauder) and one in the Routledge Sand Hills (Fig. 5.1). The fifth and westernmost population of *T. occidentalis* is located in the Pakowki Sand Hills of southeastern Alberta (Fig. 5.1).

To date, most research on Canadian populations of *T. occidentalis* has dealt with population numbers, its association with other plant species within the community, and to a limited extent, reproductive strategies. Although recent estimates regarding the size of these populations exist, the issue of genetic variation within and among populations of *T. occidentalis* remains largely unexplored.

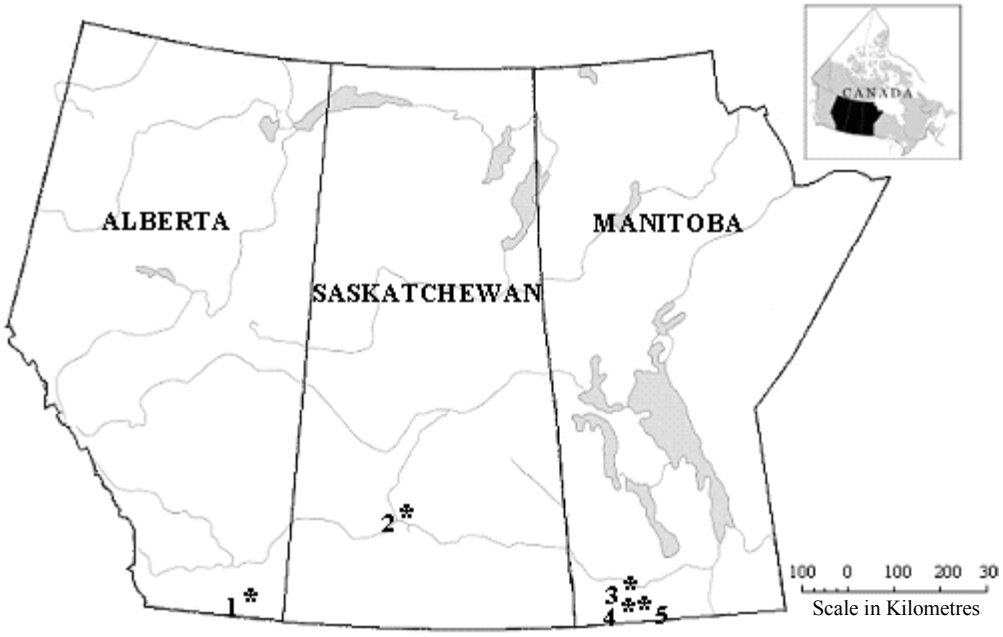


**Figure 5.1.** Map of the prairie provinces of Canada showing the general locations of the populations of *Tradescantia occidentalis*.

Since the investigation of genetic diversity provides an indication of the variability of genotypes in wild populations, such estimates are valuable in conservation biology. Several molecular methods have been used to obtain genetic diversity estimates, including AFLPs, a method that has gained popularity in studies of species at risk. AFLPs use the selective PCR amplification of restriction fragments, which involves the digestion of genomic DNA with two enzymes, followed by ligation to double stranded adapters, subsequent pre-selective and selective PCRs, and visualization on a polyacrylamide gel (Martínez-Ortega et al. 2004). The restriction endonucleases include a frequent cutter and a rare cutter. The frequent cutter has many restriction sites throughout the genome and generates small DNA fragments ideal for amplification. The rare cutter recognizes fewer restriction sites, resulting in larger fragments. Using two enzymes increases selectivity because only segments cut by both endonucleases are

amplified (Vos et al. 1995). Since the advantages and disadvantages of AFLPs have been discussed in section 2.4.1, the reader is referred to this section.

As a consequence of habitat fragmentation, global warming, and increased numbers of rare species, conservation programs are becoming more prevalent in Canada. The current nationally threatened and provincially endangered rankings of *Tradescantia occidentalis*, the small number of populations, and the minimal information regarding genetic variation, make this species an ideal candidate for this type of investigation. The primary goal of this research is to investigate levels of genetic diversity in *T. occidentalis* at the intra- and interpopulation levels in Canada. It is hypothesized that higher levels of variation exist at an interpopulation level than at the intrapopulation level due to the geographic and putative reproductive isolation among the populations.

## 5.2 Materials and Methods

### 5.2.1 Plant Material

Plant material was collected *in situ* between June and July of 2005. Leaf tissue was removed from a total of 30 individuals in each population, the maximum number granted by SERM, MCDC, and ASRD. Plants were randomly selected in the Saskatchewan population, but in the Manitoba and Alberta populations, individuals were selected within the random transects designed for the population inventory (see section 4.3.1). A distance of at least one metre between individuals was maintained in all populations. Some samples deteriorated due to warm temperatures in the field;

consequently, 20 plants per population were used for AFLP analysis in order to maintain a consistent sample size. Similar studies involving AFLP techniques have used sample sizes ranging from 20 to 75 individuals per population (Bayer 1991; Muluvi et al. 1999). Lower sample sizes are often observed in studies of wild and threatened species, while larger sample sizes are seen in agricultural studies where ample material is readily available.

Leaf samples were wrapped in damp paper towel and placed in a cooler or dried in silica gel to preserve tissue for DNA extraction. The preservation protocol followed the recommendations of Chase and Hill (1991), who state that plant tissue should be placed in silica gel in a ten to one ratio. This allows tissue to dry within 12 hours, thus preventing DNA degradation. One voucher specimen from each province was prepared for the collection of the University of Saskatchewan (SASK) herbarium.

### 5.2.2 DNA Extraction

DNA extraction followed a Mini-Prep CTAB DNA extraction protocol (Cota-Sánchez et al., in review) modified from Murray and Thompson (1980) and Saghai-Maroof et al. (1986) for mucilaginous species. Leaf tissue from live or dried tissue was frozen in liquid nitrogen and powdered with a mortar and pestle. Tissue was added to an Eppendorf tube containing hexacetyl trimethylammonium bromide (CTAB) and 2-mercaptoethanol, heated to 55-60˚C for one hour, and mixed periodically. Then, repeated organic extractions with chloroform:isoamyl alcohol (24:1) were conducted to separate cellular debris and excess mucopolysaccharides, followed by isopropanol precipitation of the supernatant at -20˚C overnight. The precipitated DNA was

centrifuged to form a pellet, which was dried and resuspended in tris ethylene diaminetetra-acetic acid (TE). The samples were incubated at 37˚C for 30 minutes with RNAase, followed by the addition of 2.5 M NaOAc and 95% ethanol for overnight precipitation at -20˚C. The resulting pellet was centrifuged, dried, and washed with 70% ethanol before resuspension in TE and storage at -20˚C. Following extraction, the total DNA was quantified using a Biophotometer UV Spectrophotometer (Eppendorf AG), stained with ethidium bromide, and electrophoresed on a 1% agarose gel against a marker of known molecular weight for visualization of the extracted product.

### 5.2.3 Amplified Fragment Length Polymorphism Analysis

DNA from 20 individuals was initially tested to select the primer combinations that yielded the highest number of polymorphisms, which are bands present in some samples but absent in others. Three of nine screened combinations were selected (Table 5.1), which is comparable with other AFLP studies using three (Gaudeul et al. 2000) or four (Muluvi et al. 1999) primer sets. Primers were tested several times on the same set of DNA samples to ensure reproducibility.

The AFLP protocol was modified from methods outlined by the genetics laboratory in the Department of Plant Sciences at the University of Saskatchewan (G. Scoles, unpubl.) and Gaudeul et al. (2000). Foremost, all DNA samples were brought to 100 ng/µl and subjected to a restriction reaction with the following components: 5.0 µl DNA, 2.5 µl One-Phor-All Buffer (10X) (Amersham Biosciences), 17.0 µl $dH_2O$, 0.25 µl *Mse*I (10 units/µl) (Fermentas), and 0.25 µl *Eco*RI (10 units/µl) (Invitrogen). The reaction was incubated at 37˚C for three hours, then cooled on ice. Next, the samples

**Table 5.1.** Adapters, pre-selective primers, and selective primer combinations used to screen genetic diversity in *Tradescantia occidentalis*. *Eco*RI primers are fluorescent labeled, E-ACT and E-ACA with FAM (blue), and E-ACC with NED (yellow).

| Primer Type | Primer Name | Oligonucleotide Sequence |
|---|---|---|
| *Eco*RI Adapter | *Eco*RI-F | CTC GTA GAC TGC GTA CC |
| | *Eco*RI-R | AAT TGG TAC GCA GTC |
| *Mse*I Adapter | *Mse*I-F | TAC TCA GGA CTC AT |
| | *Mse*I-R | CAG GAT GAG TCC TGA G |
| Pre-selective Primers | *Eco*RI | GAC TCG GTA CCA ATT CA |
| | *Mse*I | GAT GAG TCC TGA GTA AC |
| Selective Primers – 1 | E-ACT/M-CTG | E-GAC TGC GTA CCA ATT CAC T/ M-GAT GAG TCC TGA GTA ACT G |
| Selective Primers – 2 | E-ACA/M-CAG | E-GAC TGC GTA CCA ATT CAC A/ M-GAT GAG TCC TGA GTA ACA G |
| Selective Primers – 8 | E-ACC/M-CTA | E-GAC TGC GTA CCA ATT CAC C/ M-GAT GAG TCC TGA GTA ACT A |

were heated at 70˚C for 15 minutes and cooled prior to the ligation reaction. Ligation included 25.0 µl restriction enzyme digest, 24.0 µl adapter-ligation solution, and 1.0 µl T4 DNA ligase (Invitrogen). The adapter-ligation solution contained the following ingredients: 1.0 µl *Mse*I adapter (20 µM) (Invitrogen), 1.0 µl *Eco*RI adapter (2 µM) (Invitrogen), 0.96 µl ATP (10 mM) (Fermentas), 2.4 µl One-Phor-All Buffer, and 18.64 µl $dH_20$. The ligation reaction was conducted at 20˚C for two hours then placed on ice. Samples were diluted ten times in T0.1E buffer and stored at -20˚C. The T0.1E buffer was made of 1 mL 1.0 M Tris pH 8.0, 20 µl 0.5 M EDTA pH 8.0, and 98.9 mL $dH_20$.

The pre-selective PCR was performed with the following components: 2.5 µl diluted ligation template, 40 µl pre-selective primer mix, 5 µl 10X *Taq* buffer, 1.5 µl 50 mM $MgCl_2$, 0.2 µl *Taq*, and 0.8 µl $dH_2O$. The pre-selective primer mix included 10 µl *Mse*I pre-selective primer (300 ng/µl) (Invitrogen), 10 µl *Eco*RI pre-selective primer (300 ng/µl) (Invitrogen), 800 µl dNTP (1.25 mM each) (Invitrogen), and 3180 µl $dH_2O$. Sequences of the pre-selective primers are provided in Table 5.1. The conditions of the pre-selective PCR were as follows: denaturing for 30s at 94˚C, annealing for 60s at

56˚C, and extension for 60s at 72˚C for 20 cycles. The reactions were held at 4˚C upon completion. Products of the pre-selective PCR were diluted 50 times in T0.1E Buffer and stored at -20˚C until use in the selective PCR.

The selective PCR mixture included the following components: 2.5 µl diluted pre-selective product, 2.0 µl 10X *Taq* buffer, 0.6 µl 50 mM $MgCl_2$, 1.0 µl fluorescent-labeled *Eco*RI primer (Applied Biosystems), 1.0 µl *Mse*I primer (Applied Biosystems), 3.5 µl dNTP, 0.2 µl *Taq*, and 9.2 µl $dH_2O$. The sequences of the selective primers are provided in Table 5.1. Reaction conditions for the selective PCR were as follows: denaturing for 60s at 94˚C, annealing for 60s at 65˚C, and extension for 90s at 72˚C, repeated for ten cycles, decreasing the annealing temperature by 1˚C per cycle, followed by 23 cycles of denaturing for 30s at 94˚C, annealing for 30s at 56˚C, and elongation for 60s at 72˚C. Reactions were held at 4˚C upon completion.

Products of the selective PCR were visualized in an automated ABI Prism 377 DNA Sequencer (Applied Biosystems) using GeneScan software v.3.1. A polyacrylamide gel composed of 13.5 g urea, 19.5 mL $dH_20$, 3.75 mL Long Ranger (50%) (Cambrex Biosciences), and 3.75 mL 10X TBE was prepared to visualize the AFLP products. A proportion (0.7 µl) of selective PCR product was added to 1.7 µl loading buffer. A total of 2.0 µl was loaded into a polyacrylamide gel and electrophoresed in the automated sequencer for 2.5 h with the GeneScan software.

### 5.2.4 Data Analysis

The raw data generated by GeneScan included chromatograms and tables summarizing the peak size, height, and area. Chromatograms were chosen over band

images as a scoring system because of the time and computer programs required to convert GeneScan results to images. In addition, it was decided that the ratio of signal to interference associated with chromatograms would be equal in the band images. The peaks were analyzed, scored as present or absent, and entered into a Microsoft Excel matrix. For consistency, band patterns from several gels were compared and peaks were numbered according to their relative position and pattern. The size, height, and area of peaks on the chromatograms could not be used to score the bands because these characters vary with gel conditions. Ambiguous bands, i.e. those that were very large or only observed a few times, were not scored because their presence or absence was attributed to lack of template or interference on the gel. The minimum height of bands scored varied according to the amount of signal and height of known peaks, but generally peaks with a height less than 200 units were not scored. Because all samples could not be accommodated on the same gel and some had to be electrophoresed more than once to obtain comparable results, several gels were prepared for each primer.

Statistical analysis was performed to compare intra- and interpopulation diversity. Based on presence or absence of bands, genetic similarity dendrograms were created using sequential, agglomerative, hierarchical, and nested (SAHN) clustering methods in NTSYS-pc version 1.80 (Rohlf 1992). Dendrograms were constructed for each population and primer using the Dice similarity coefficient and the complete and unweighted pair group method with arithmetic mean (UPGMA) clustering methods. The UPGMA method creates a distance matrix for the smallest distance element and joins operational taxomonic units (OTUs) at an internal node assuming equal rates of evolution along all dendrogram branches (Avise 1994). Both the UPGMA and NJOIN

methods assume that characters are homologous and independent (Avise 1994). The dissimilarity matrices were also used for principal coordinate analysis (PCOORDA), which was carried out using the DCENTER and EIGEN programs of NTSYS. Dendrograms were created for each population and the combination of the five populations using the three individual primers as well as for combined primers and populations.

In the remaining statistical analysis involving NTSYS, band frequency data was calculated as the occurrence of a particular band in a population. This data was used to create genetic distance matrices in SIGMEND, which computes matrices of genetic distance coefficients, for each primer and for the combination of primers using the NEI172 coefficient, a measure of Nei's genetic distance. Dendrograms and neighbour joining trees were created in SAHN and NJOIN, respectively. The NJOIN method produces estimated phylogenetic trees based on Saitou and Nei's neighbour joining method and allows for unequal rates of evolution among branches by constructing a transformed distance matrix at each step of the analysis (Avise 1994). For primers one, two, and the combined primers, the trees were weighted using the midpoint joining method; however, this method resulted in error messages for primer eight. As a result, these trees were unweighted and the Alberta population was chosen as the outgroup because it is the most geographically distant from the other Canadian and American populations of *Tradescantia occidentalis*. It is recognized that this choice may have some effect on the placement of the Alberta population relative to the other Canadian populations and that accessions from the United States of America would have been a better alternative for rooting purposes. However, representative samples from the

United States were not available for study. As a result, the interpretation of the interpopulation results largely excludes primer eight. ArcGIS based plots created in ArcGIS v.9.1 were obtained through GIS Services of the University of Saskatchewan for comparison of dendrogram topology and geographic distribution within populations.

## 5.3 Results

A total of 62 of 83 (75%) reliable bands detected with the three primer sets were polymorphic. The highest numbers of polymorphic bands were observed in the three Manitoba populations, while lower values prevailed in Saskatchewan and Alberta (Table 5.2).

**Table 5.2.** Number and percentage of polymorphic bands detected with three primers in the five populations of *Tradescantia occidentalis.*

| Population | Saskatchewan | Manitoba | | | Alberta | All Populations |
|---|---|---|---|---|---|---|
| | | Routledge | MHHC | Lauder | | |
| # Bands | 28 (33%) | 49 (59%) | 59 (71%) | 43 (52%) | 34 (41%) | 62 (75%) |

### 5.3.1 Intrapopulation Diversity

The dendrograms obtained in the SAHN analysis show different relationships among samples for each primer. Only SAHN dendrograms using the complete clustering method and Dice's coefficient are considered here because the relationships depicted in the trees obtained using the UPGMA method were unresolved. The number of boxes on the terminal dendrogram branches is equal to the number of geographical subpopulations within a population. For example, in Saskatchewan, there are four subpopulations (PFRA north, PFRA south, Highway 19, and Douglas) (Fig. 5.2E);

therefore the dendrograms were arbitrarily grouped into four clusters (Fig. 5.2A-D). This was purposely done in each population to test whether the individuals form clusters as part of the dendrogram topology matched the geographic distribution of individuals in the ArcGIS plots.

#### 5.3.1.1 Saskatchewan Population

Based on three primers, the levels of genetic similarity in the Saskatchewan population range from 0.64 to 1.00 (Fig. 5.2A-D). Primer two shows the lowest similarity level (0.64) (Fig. 5.2B), while primer one and the combined primers provide the greatest distinction among individuals with similarity coefficients of 0.84 and 0.85, respectively (Fig. 5.2A, D). The highest number (17) of genetically identical individuals is observed with primer eight (Fig. 5.2C), but several accessions also exhibit genetic similarity of 1.00 with each primer. The hypothesis that the four subpopulations in the Saskatchewan population are genetically different can be tested based on dendrogram topology. The clusters obtained with the AFLP data do not support the geographic plot of the accessions of *Tradescantia occidentalis* (Fig. 5.2E); thus indicating that at the genetic level, the four spatially separated subpopulations form a single, relatively uniform entity.

#### 5.3.1.2 Routledge (Manitoba) Population

The genetic similarity coefficients in the Routledge population range from 0.48 (primer eight) to 0.63 (primer two) to 0.72 (combined primers) to 0.87 (primer one)

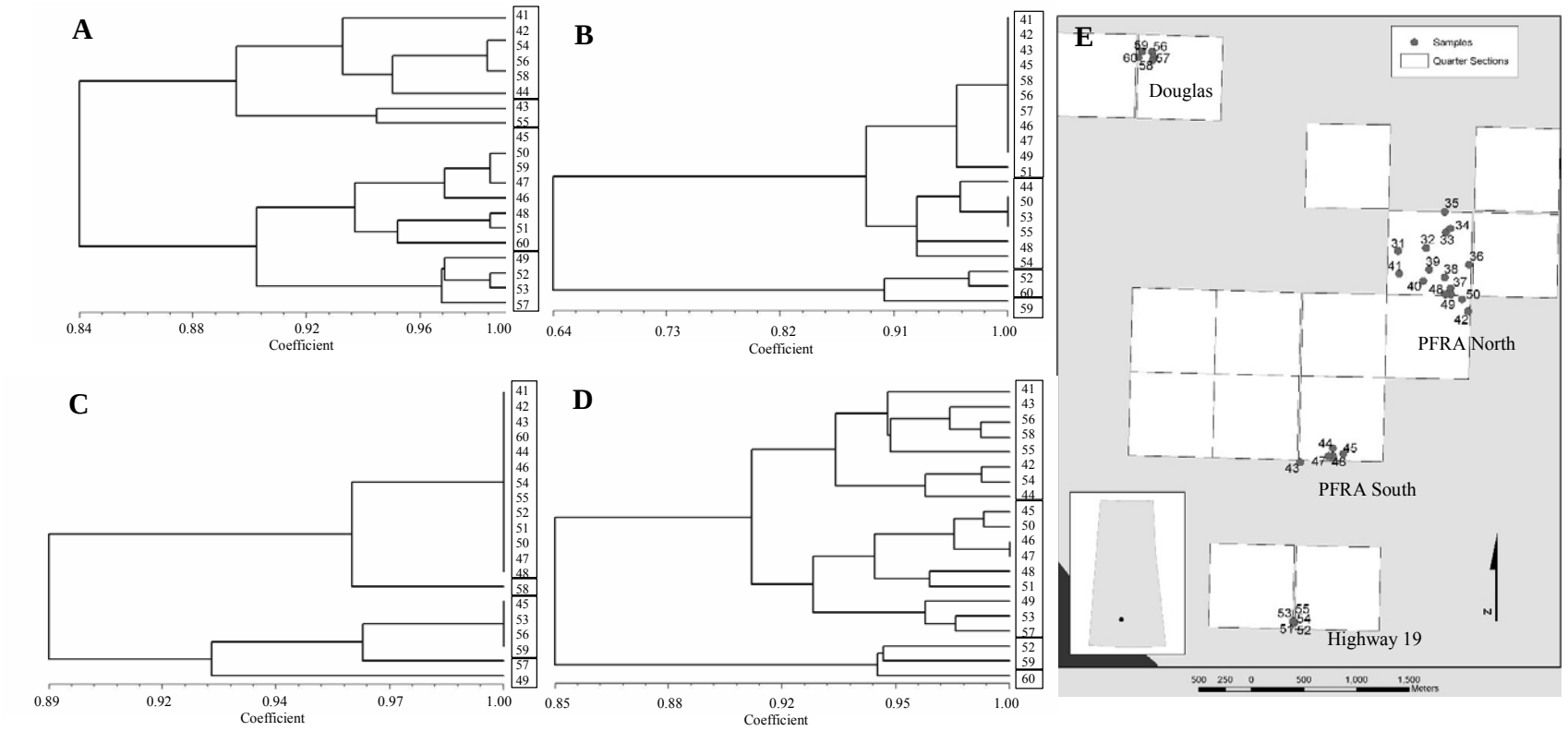


**Figure 5.2.** Dendrograms showing genetic similarity in SAHN using complete clustering and an ArcGIS based plot for the Saskatchewan population of *Tradescantia occidentalis*. **A**. Primer one. **B**. Primer two. **C**. Primer eight. **D**. Combined primer sets one, two and eight. **E**. ArcGIS based plot showing the four subpopulations and geographic distribution of individuals.

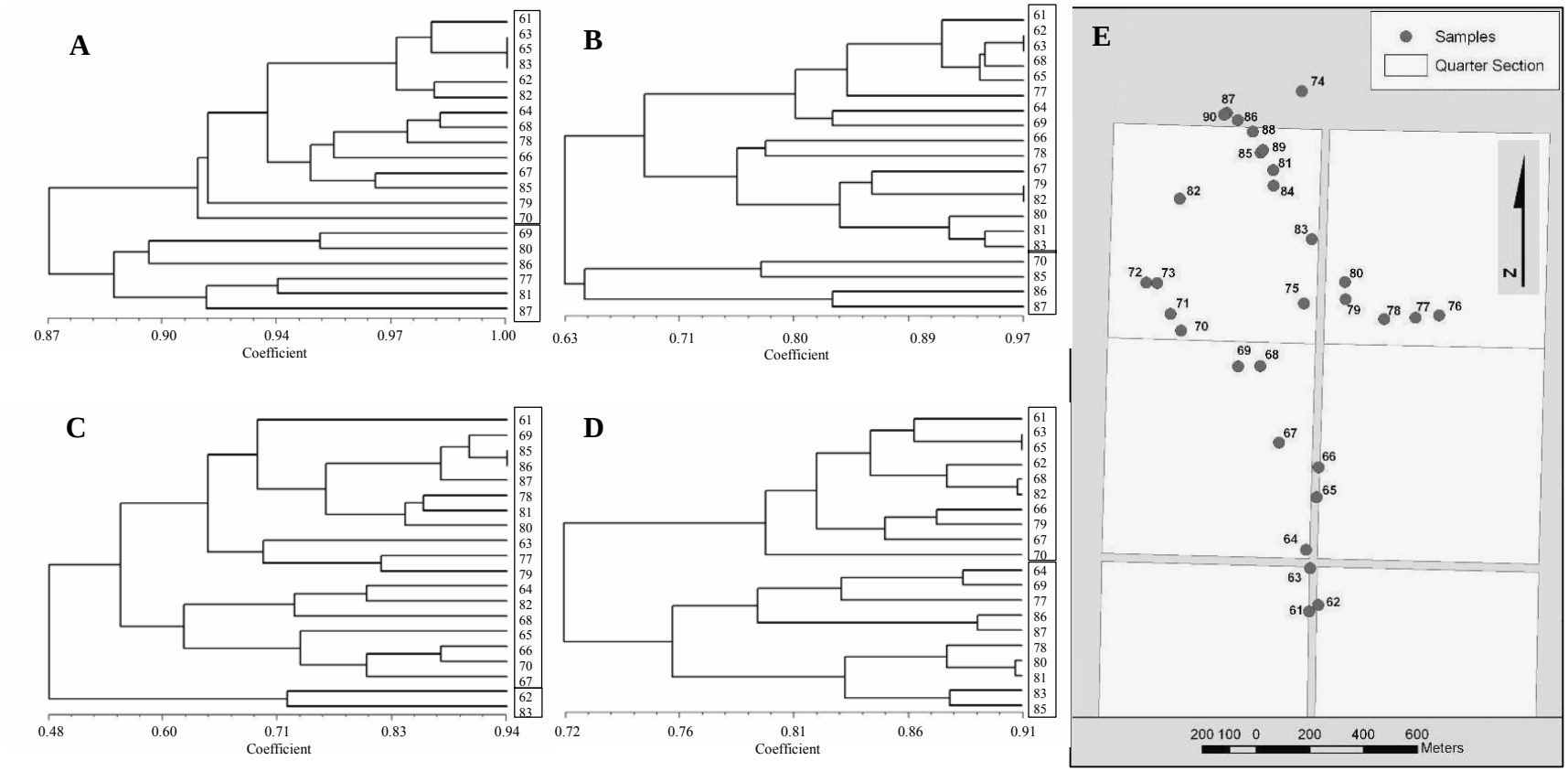


**Figure 5.3.** Dendrograms showing genetic similarity in SAHN using complete clustering and ArcGIS based plot for the Routledge (Manitoba) population of *Tradescantia occidentalis*. **A**. Primer one. **B**. Primer two. **C**. Primer eight. **D**. Combined primer sets one, two, and eight. **E**. ArcGIS based plot showing the geographic distribution of individuals.

(Fig. 5.3A-D). The maximum number of individuals with a genetic similarity index of 1.00 is three with primer one, which is lower than the Saskatchewan population. The hypothesis that the individuals on the two sand hill formations are genetically distinct can be tested in this population. Indeed, clustering of individuals within the dendrograms is in two major groups (Fig. 5.3A-D); however, the ArcGIS plot of the accessions of *Tradescantia occidentalis* (Fig. 5.3E) are not congruent with the clades based on genetic similarity. Thus, it can be said that the Routledge population, distributed over the two sand ridges, is one population in terms of genetic similarity, even though a relatively large number of polymorphic sites and high levels of genetic diversity are detected.

#### 5.3.1.3 MHHC (Manitoba) Population

The levels of genetic similarity in the MHHC population are the lowest among all of the Canadian populations, ranging from 0.40 (primer eight) to 0.67 (primer one) to 0.69 (primer two and combined) (Fig. 5.4A-D). The highest number (13) of genetically identical individuals is seen with primer two (Fig. 5.4B). It is difficult to propose a hypothesis regarding the genetic distinctness of clusters in this population because of the small number of individuals and the area to which this population is restricted. Both the dendrogram and the ArcGIS based plot show individuals divided into two broad groups; however, these clusters are not congruent with each other (Fig. 5.4A-E). The highest levels of polymorphisms are found in the MHHC population, which is an unexpected result because of the small size of this population. Generally, smaller populations are expected to have lower levels of polymorphisms due to inbreeding and genetic drift.

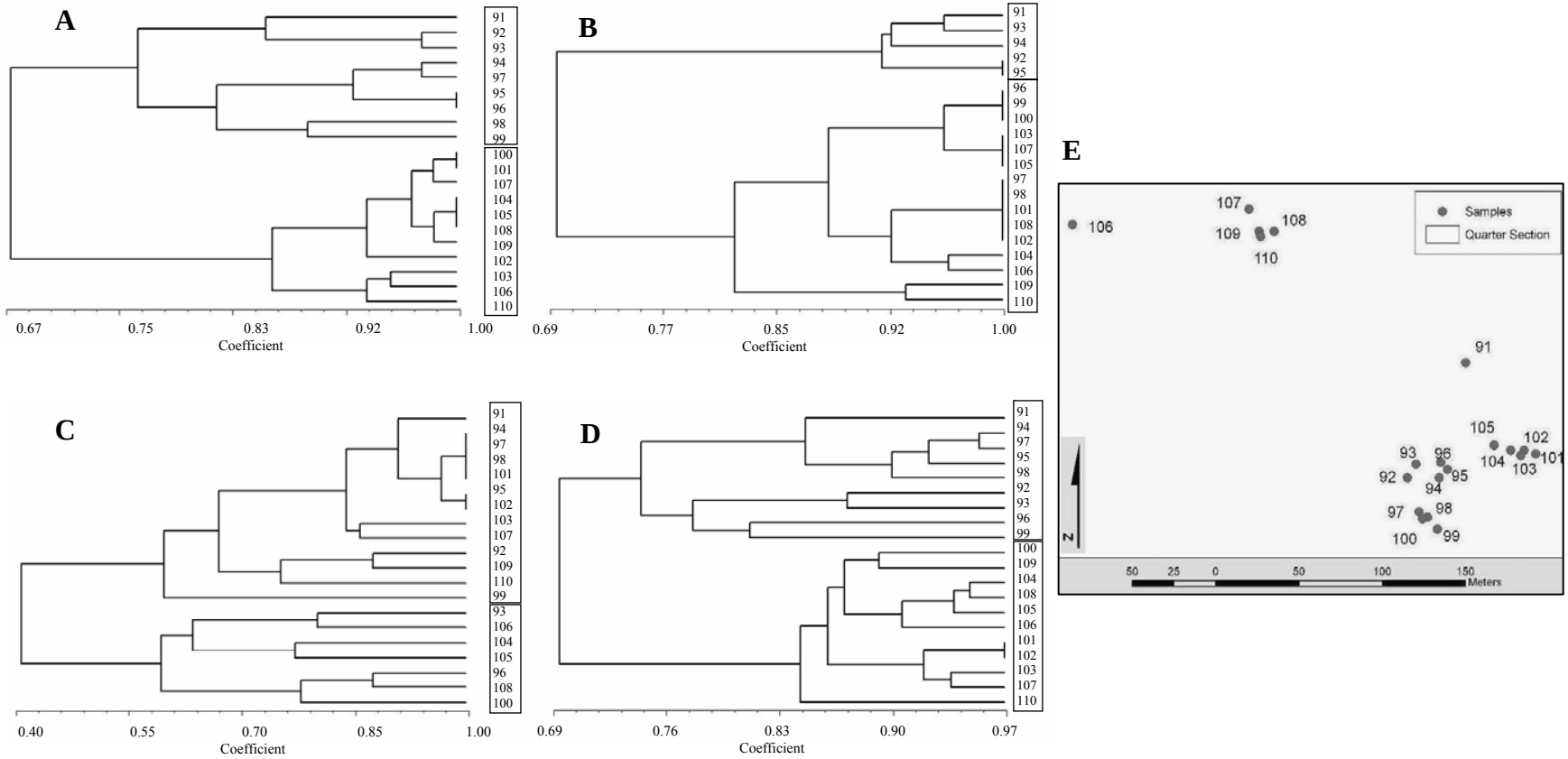


**Figure 5.4.** Dendrograms showing genetic similarity in SAHN using complete clustering for the MHHC (Manitoba) population of *Tradescantia occidentalis*. **A**. Primer one. **B**. Primer two. **C**. Primer eight. **D**. Combined primer sets one, two and eight. **E**. ArcGIS based plot showing the geographic distribution of individuals in the MHHC population.

#### 5.3.1.4 Lauder (Manitoba) Population

Relatively high levels of genetic diversity were also observed in the Lauder population. The values of genetic similarity range from 0.48 (primer eight) to 0.62 (primer one) to 0.68 (combined) to 0.74 (primer two) (Fig. 5.5A-D). The highest number (11) of genetically identical samples is seen with primer two (Fig. 5.5B). It can be hypothesized that individuals on opposite ends of the dune are less genetically similar than individuals that are physically closer. In spite of the small population size, a high number of polymorphisms are present and the dendrograms obtained from the analyses of this population separate individuals into two main clusters. Nonetheless, the geographic distribution of accessions of *Tradescantia occidentalis* plotted in ArcGIS based maps follows the shape of the dune formation. This is incongruent with the dendrogram clades (Fig. 5.5A-D), which show no evident separation of the population into subgroups (Fig. 5.5E).

#### 5.3.1.5 Alberta Population

Analyses of the AFLP data show that Alberta population has the lowest genetic variation among the five Canadian populations. This is a reflection of the lower number of polymorphic sites. The indices range from 0.73 (primers two and eight) to 0.84 (combined) to 0.85 (primer one) (Fig. 5.6A-D). The highest number (11) of individuals with 100% genetic similarity is observed with primer two (Fig 5.6B). Like in the Lauder (Manitoba) population, based on the local distribution of the Alberta population, it can be hypothesized that individuals located at opposite ends of the population will be less similar genetically than closely situated plants. An ArcGIS plot of the accessions of

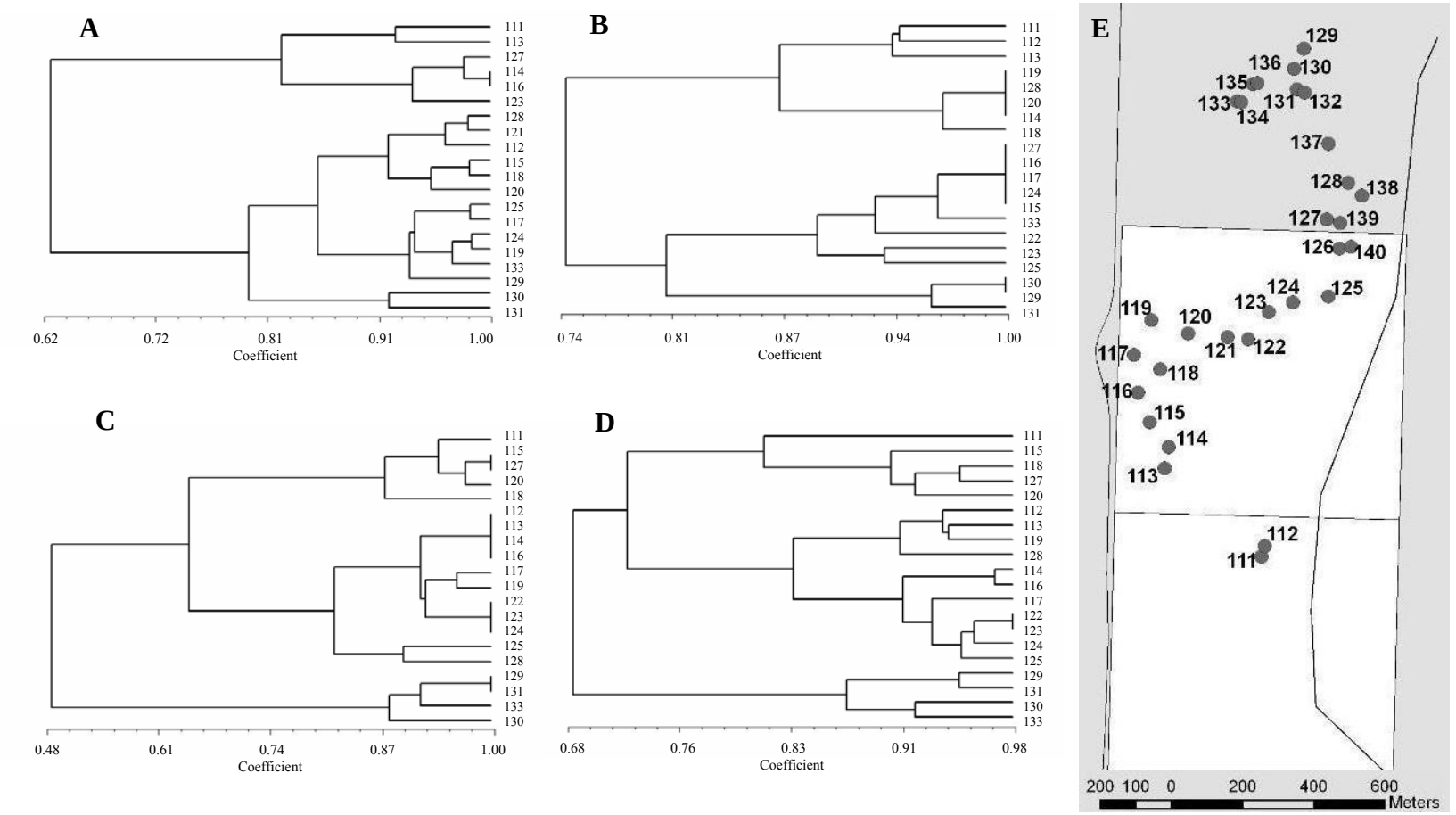


**Figure 5.5.** Dendrograms showing genetic similarity in SAHN using complete clustering and an ArcGIS based plot for the Lauder (Manitoba) population of *Tradescantia occidentalis*. **A**. Primer one. **B**. Primer two. **C**. Primer eight. **D**. Combined primer sets one, two, and eight. **E**. ArcGIS based plot showing the geographic distribution of individuals.

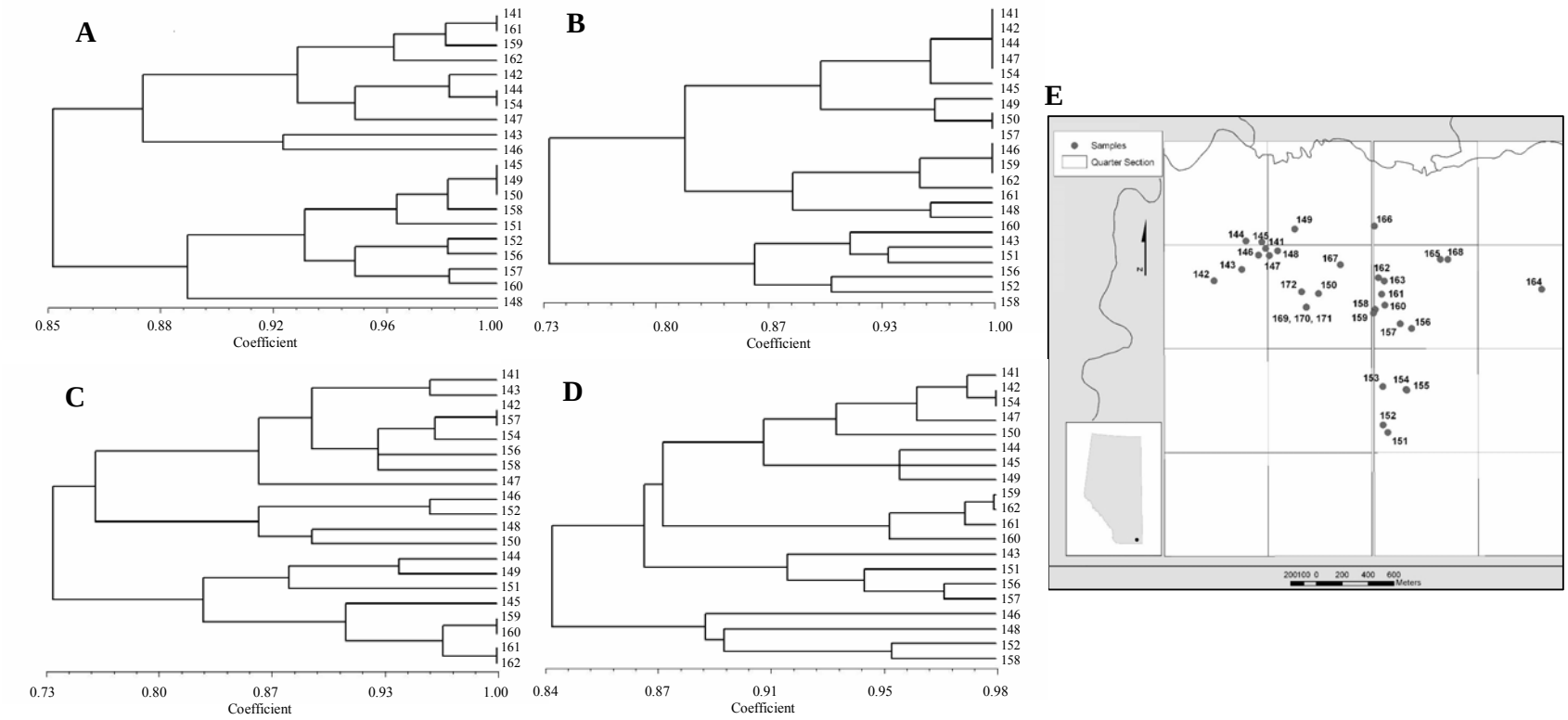


**Figure 5.6.** Dendrograms showing genetic similarity in SAHN using complete clustering and an ArcGIS based plot of the Alberta population of *Tradescantia occidentalis*. **A**. Primer one. **B**. Primer two. **C**. Primer eight. **D**. Combined primer sets one, two, and eight. **E**. ArcGIS based plot showing the geographic distribution of individuals.

*Tradescantia occidentalis* in the Alberta population shows a scattered distribution (Fig. 5.6E), which is discordant with the dendrogram topologies (Fig. 5.6A-D).

### 5.3.2 Interpopulation Diversity

Interpopulation diversity is important in conservation genetics because it shows the extent to which populations have differentiated. Based on the combined analyses including the data set from the individual and combined primers of the five populations, the following inferences can be made. First, although the individual primers do not provide enough information to separate all of the Canadian populations from each other, primers one and two group 35% and 55% of the individuals within the MHHC (Fig. 5.7) and Lauder (Fig. 5.8) populations, respectively. Likewise, the polymorphisms generated with primers one (Fig. 5.7) and two (Fig. 5.8) distinguish 45% and 85% of the individuals in the Routledge population. Similarly, even though primer eight does not clearly differentiate among all populations, it distinguishes 70%, 40%, and 35% of individuals from the Saskatchewan, Alberta, and MHHC populations (Fig. 5.9), in that order.

On the other hand, the additive effect of the number of polymorphic sites is evident in the combined analysis, in which 100%, 90%, and 85% of the Alberta, Routledge, and Saskatchewan populations form separate clusters. This indicates their genetic distinctness (Fig. 5.10), which can be explained due to the higher levels of genetic diversity. However in this analysis, only 62.5% of MHHC and Lauder samples cluster together (Fig. 5.10). Overall, the combined analysis integrates the highest

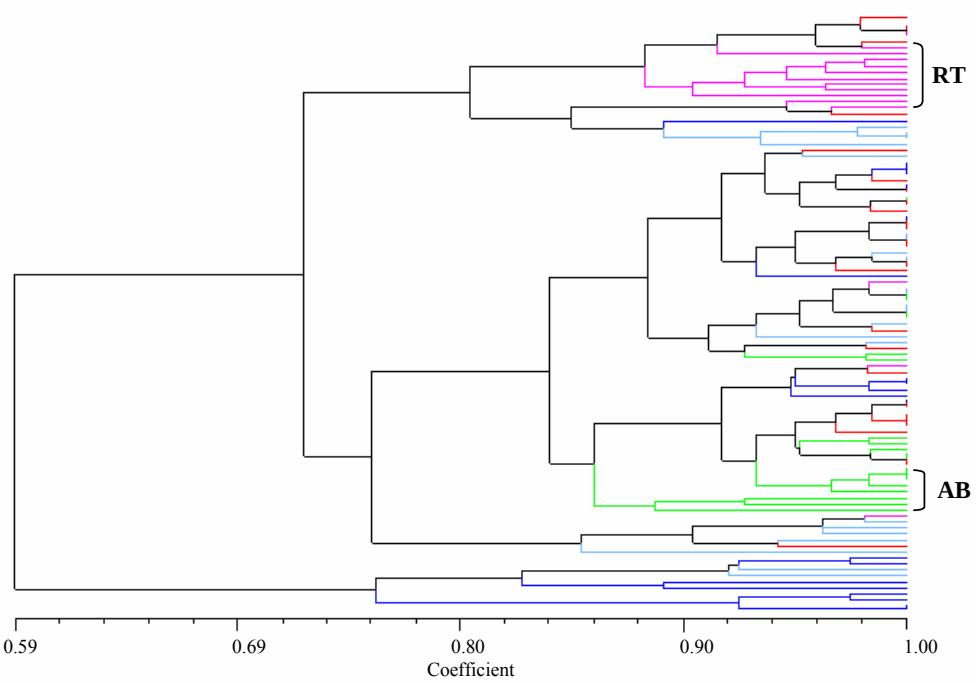


**Figure 5.7.** Dendrogram showing genetic similarity in SAHN using complete clustering for primer one. The analysis includes accessions from the five Canadian populations of *Tradescantia occidentalis*. Red = Saskatchewan (SK), Pink = Routledge (RT), Dark Blue = MHHC (MH), Light Blue = Lauder (LD), Green = Alberta (AB).

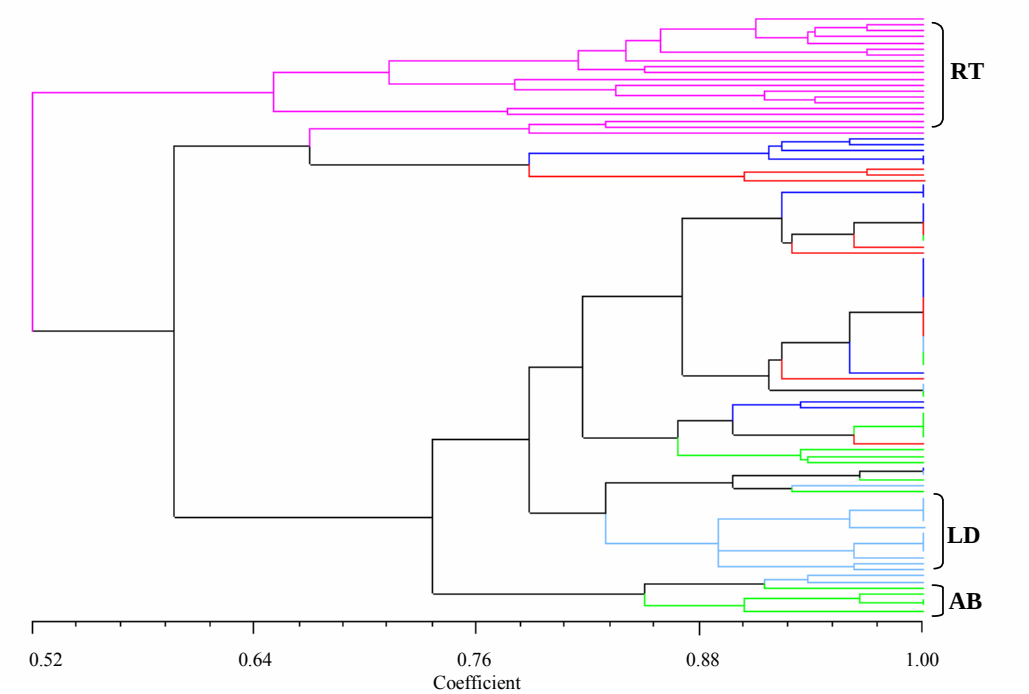


**Figure 5.8.** Dendrogram showing genetic similarity in SAHN using complete clustering for primer two. The analysis includes accessions from the five populations of *Tradescantia occidentalis*. Red = Saskatchewan (SK), Pink = Routledge (RT), Dark Blue = MHHC (MH), Light Blue = Lauder (LD), Green = Alberta (AB).

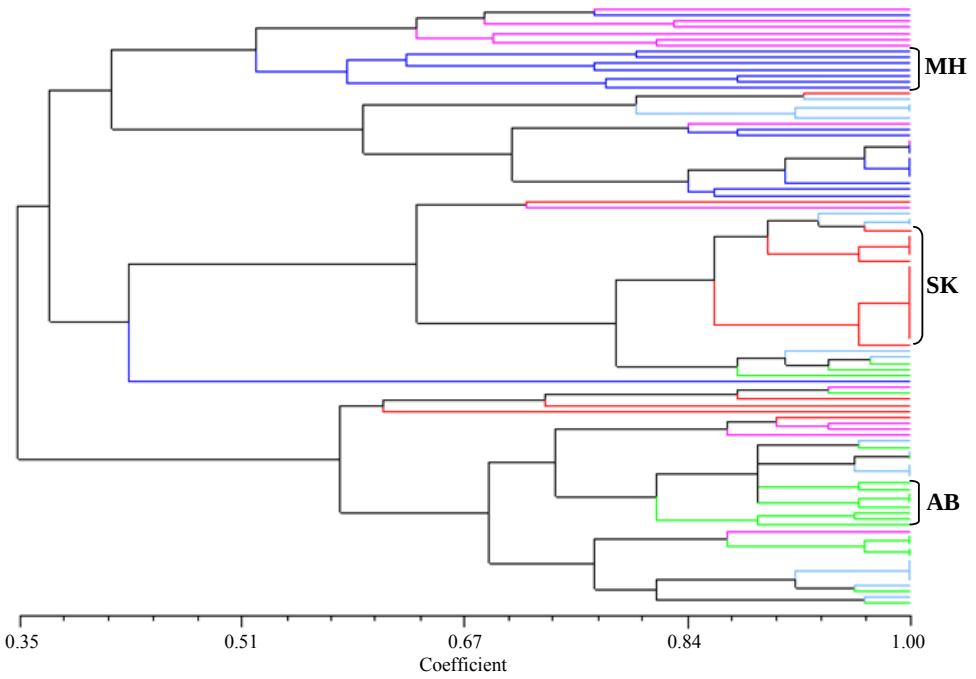


**Figure 5.9.** Dendrogram showing genetic similarity in SAHN using complete clustering for primer eight. The analysis includes accessions from all the five Canadian populations of *Tradescantia occidentalis*. Red = Saskatchewan, Pink = Routledge, Dark Blue = MHHC, Light Blue = Lauder, Green = Alberta.

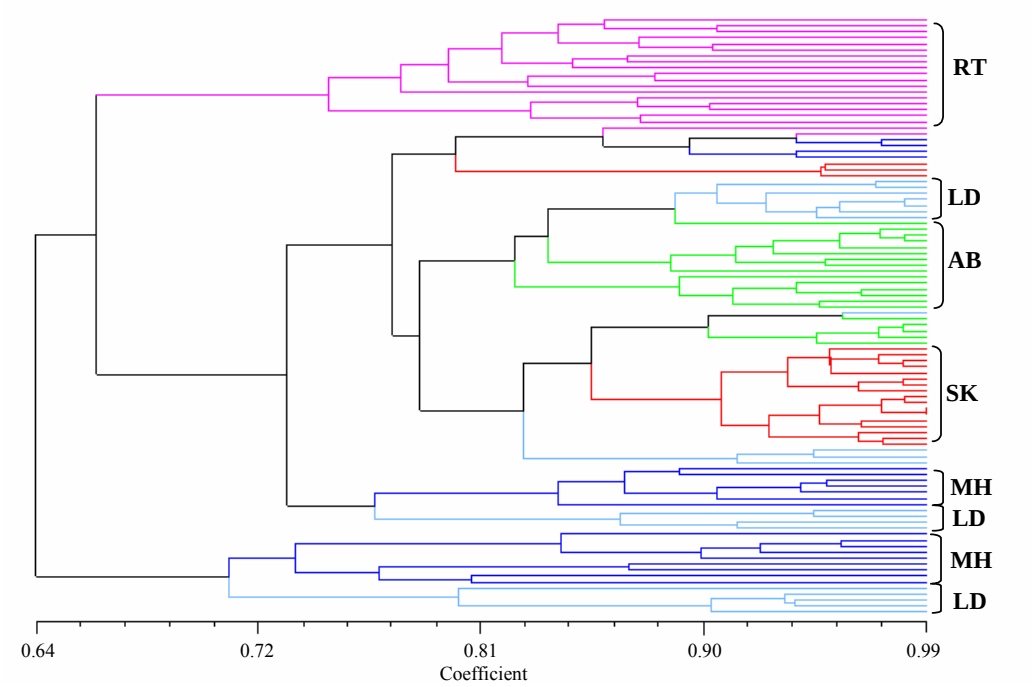


**Figure 5.10.** Dendrogram showing genetic similarity in SAHN using complete clustering for combined primers. The analysis includes the accessions from the five Canadian populations of *Tradescantia occidentalis*. Red = Saskatchewan, Pink = Routledge, Dark Blue = MHHC, Light Blue = Lauder, Green = Alberta.

number of polymorphisms, which provides the most informative clustering of individual populations to infer their degree of genetic relationship.

Based on the dendrograms obtained from the SAHN analysis, primer one suggests that the three Manitoba populations are the most genetically similar, forming a separate cluster from that of the Saskatchewan and Alberta populations (Fig. 5.11A). In the same way, the analyses of primer two and combined primers indicate that the Saskatchewan and Alberta populations are more closely related to the Lauder and MHHC populations than to the Routledge population (Figs. 5.11B, D). Further, the small genetic distances indicate the proximity of the Manitoba populations to each other relative to the Saskatchewan and Alberta populations (Table 5.3). The genetic distance matrices obtained for primers one, two, eight, and the combined primers are provided in Tables 5.3, 5.4, 5.5, and 5.6, respectively, and suggest that the Manitoba populations are more closely related.

**Table 5.3.** Nei's genetic distance for primer one calculated in SIGMEND below the diagonal and approximate geographic distance (km) between Canadian populations of *Tradescantia occidentalis* above the diagonal.

| Population | Routledge | MHHC | Lauder | Alberta | Saskatchewan |
|---|---|---|---|---|---|
| **Routledge** | | 83 | 81 | 804 | 524 |
| **MHHC** | 0.04030 | | 2.5 | 868 | 460 |
| **Lauder** | 0.02983 | 0.02050 | | 868 | 460 |
| **Alberta** | 0.06814 | 0.04662 | 0.03565 | | 519 |
| **Saskatchewan** | 0.05478 | 0.02817 | 0.02323 | 0.01224 | |

**Table 5.4.** Nei's genetic distance calculated in SIGMEND for primer two among Canadian populations of *Tradescantia occidentalis*.

| Population | Routledge | MHHC | Lauder | Alberta | Saskatchewan |
|---|---|---|---|---|---|
| **Routledge** | 0.00000 | | | | |
| **MHHC** | 0.15019 | 0.00000 | | | |
| **Lauder** | 0.19921 | 0.044241 | 0.00000 | | |
| **Alberta** | 0.16608 | 0.044189 | 0.05144 | 0.00000 | |
| **Saskatchewan** | 0.15547 | 0.003388 | 0.04411 | 0.04452 | 0.00000 |

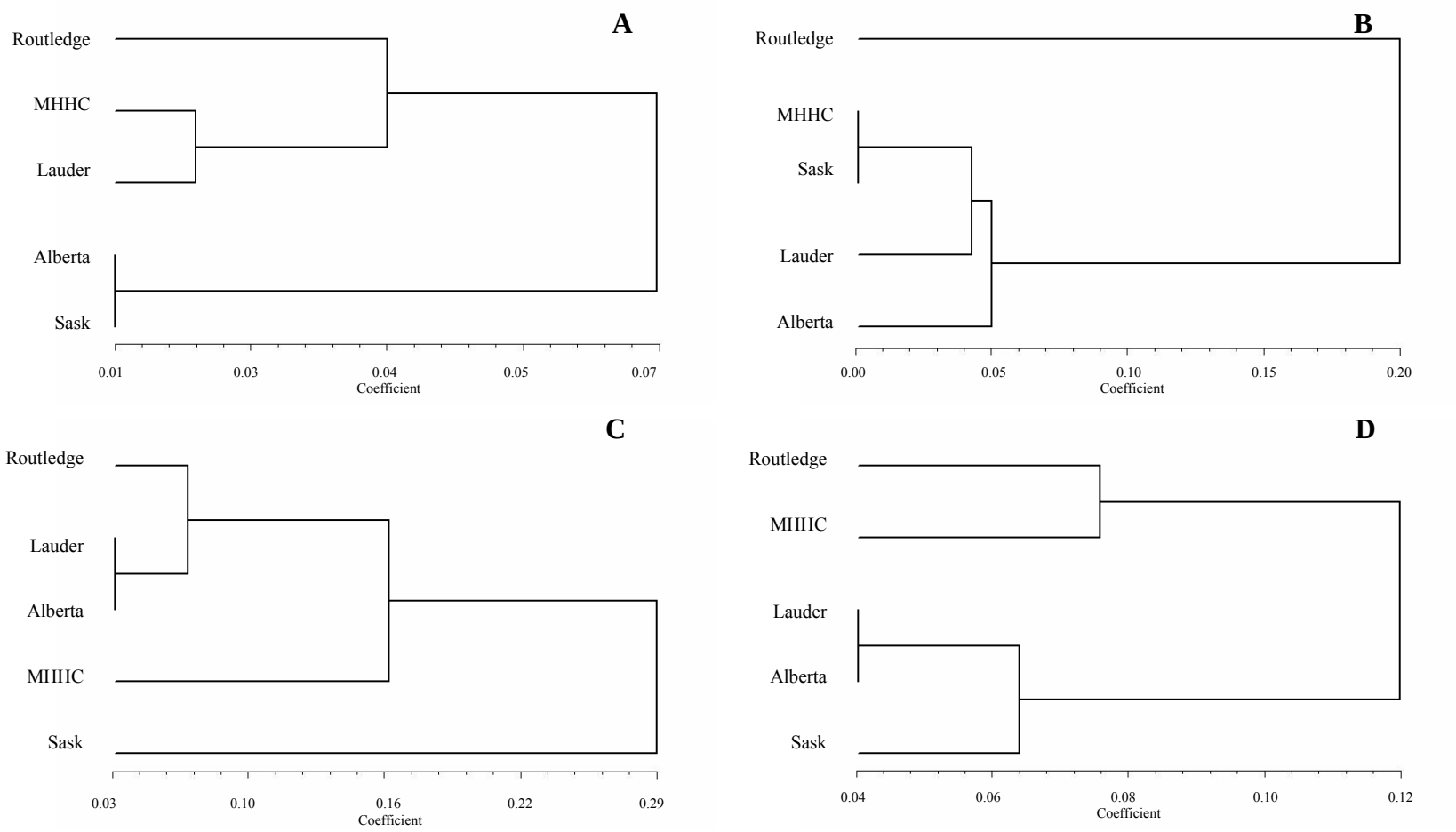


**Figure 5.11.** SAHN dendrograms using Nei's genetic distance and complete clustering for the five Canadian populations of *Tradescantia occidentalis*. **A**. Primer one. **B**. Primer two. **C**. Primer eight. **D**. Combined primer sets one, two and eight.

**Table 5.5.** Nei's genetic distance calculated in SIGMEND for primer eight among Canadian populations of *Tradescantia occidentalis*.

| Population | Routledge | MHHC | Lauder | Alberta | Saskatchewan |
|---|---|---|---|---|---|
| **Routledge** | 0.00000 | | | | |
| **MHHC** | 0.07506 | 0.00000 | | | |
| **Lauder** | 0.06612 | 0.13779 | 0.00000 | | |
| **Alberta** | 0.06822 | 0.16118 | 0.03258 | 0.00000 | |
| **Saskatchewan** | 0.24867 | 0.28674 | 0.18244 | 0.21746 | 0.00000 |

**Table 5.6.** Nei's genetic distance calculated in SIGMEND for combined primers among Canadian populations of *Tradescantia occidentalis*.

| Population | Routledge | MHHC | Lauder | Alberta | Saskatchewan |
|---|---|---|---|---|---|
| **Routledge** | 0.00000 | | | | |
| **MHHC** | 0.07185 | 0.00000 | | | |
| **Lauder** | 0.08168 | 0.05280 | 0.00000 | | |
| **Alberta** | 0.09340 | 0.07220 | 0.03922 | 0.00000 | |
| **Saskatchewan** | 0.12415 | 0.07906 | 0.06452 | 0.06272 | 0.00000 |

The neighbour joining trees also support the relationships obtained using the SAHN method. Primer one provides the most logical assessment of the putative genetic relationships among the populations. For example, the Manitoba populations form a single cluster separated from the Alberta and Saskatchewan entities (Figs. 5.11A, 5.12A). Primers two and the combined primers show the Lauder population to be more closely related to those of Alberta and Saskatchewan, followed by MHHC and Routledge (Fig. 5.12B-D). This is comparable with the relationships obtained from the SAHN dendrograms based on Nei's genetic distance (Fig. 5.10). The apparently close relationship between the Alberta and Saskatchewan populations is also supported by both analyses (Fig. 5.10). Finally, a principal coordinate analysis (PCOORDA) of the *Tradescantia occidentalis* AFLP data was performed to compare genetic relationships. However, the results from this analysis are excluded because they were not informative.

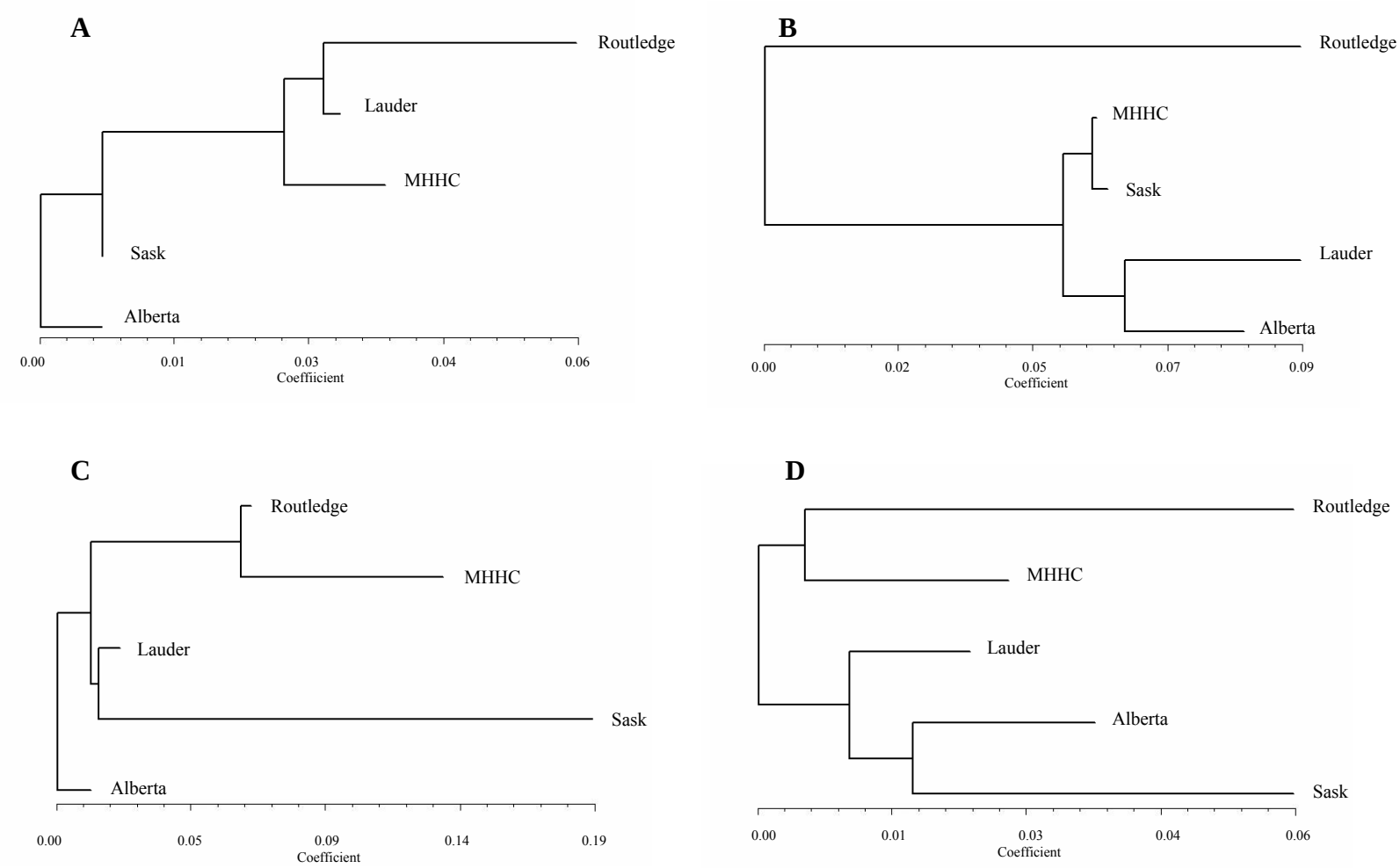


**Figure 5.12.** Neighbour joining trees using Nei's genetic distance for the five Canadian populations of *Tradescantia occidentalis*. **A**. Primer one. **B**. Primer two. **C**. Primer eight. **D**. Combined primer sets one, two and eight.

## 5.4 Discussion

In plants there appears to be a wide range in the proportion of polymorphic AFLP loci per population, from a low of 4.2% in *Helianthus annuus* L. (Asteraceae) (Hongtrakul et al. 1997) up to 85% in *Poa arachnifera* Torr. (Poaceae) (Renganayaki et al. 2001). For *Tradescantia occidentalis*, the percentage of polymorphism within a population ranges from 33% in Saskatchewan to 71% in the MHHC population (Table 5.2). This illustrates that the level of polymorphisms detected in this study is analogous to other AFLP data in plants. Similarly, the total number of polymorphic bands among the five populations of *T. occidentalis* is 62 (75%) (Table 5.2), an estimate that is comparable to other AFLP studies of genetic diversity showing polymorphism rates between 56% in the Salicaceae (Winfield et al. 1998) and 91% in the Apiaceae (Gaudeul et al. 2000).

### 5.4.1 Intrapopulation Diversity

The levels of intrapopulation genetic diversity obtained with Dice's similarity coefficient in all populations of *Tradescantia occidentalis* ranged from 0.40-1.00. Although this type of data is not available in the literature for *Tradescantia* or other commelinoid taxa, comparable levels have been reported in wild plants in the Fabaceae (Travis et al. 1996) and Palmaceae (Perera et al. 1998). Only two individuals belonging to the Saskatchewan population appeared to be genetically identical based on data of the combined primers. This finding is relevant because it demonstrates that AFLPs have sufficient resolution to distinguish individuals in the same population and that low levels of diversity observed are not due to the inability of the combined primers to detect variation.

Levels of genetic similarity in the Saskatchewan and Alberta populations are relatively high and range from 0.64-1.00 (Fig. 5.2A-D) and 0.73-1.00 (Fig. 5.6A-D), respectively. Similarly, low levels of genetic diversity have been reported in the Theaceae (Paul et al. 1997). The null hypothesis that individuals in the four subpopulations of *Tradescantia occidentalis* in Saskatchewan are genetically distinct is rejected based on analysis of the AFLP data (Fig. 5.2A-E). Likewise, the null hypothesis that individuals in the Alberta population isolated by distance are more genetically distinct is also rejected based on the analysis of AFLP data. In the Alberta population, the clusters do not show a relationship with the distribution of individuals on the landscape (Fig. 5.6A-E). Individuals separated by over a kilometre (i.e. accessions 142 and 154) appear to be more genetically similar to each other than individuals less than 200 m apart (i.e. 146 and 148) based on data from primer one and the combined analysis (Fig. 5.6A, D).

Neither population appears to be well structured, but rather each reflects panmixia, the free exchange of genetic material through outcrossing of individuals within a population. In Saskatchewan, the area separating Douglas, PFRA north and south, and Highway 19 contains suitable habitat for *Tradescantia occidentalis* (K. Remarchuk, pers. obs.), which may mean that subpopulations of this species remain undiscovered. In both Saskatchewan and Alberta, this means that although there is relatively little intrapopulation genetic diversity, pollen or propagule transfer occurs, even in the most distantly separated areas. Alternatively, in Saskatchewan the separation could have been too recent to detect any differentiation among subpopulations.

The values of Dice's similarity coefficients indicate relatively low levels of genetic diversity among individuals of the same population. It is plausible that these

populations have been separated from a larger continuous distribution, in the United States for example, for a longer period of time than the Manitoba populations, and the low levels of genetic diversity are perhaps a consequence of inbreeding and genetic drift. In fact, it has been hypothesized that a species isolated in small patches that are distributed over a wide geographic range will experience genetic drift and differentiation (Godt and Hamrick 1993).

Genetic drift, the random change in allelic frequency over time as a result of chance (Beebee and Rowe 2004), causes a decrease in intrapopulation variability and an increase in interpopulation differentiation (Ellstrand and Elam 1993). However, through gradual inbreeding, natural selection tends to remove recessive lethal alleles expressed as homozygotes without compromising the viability of a population (Lande 1999). Both inbreeding and genetic drift are natural processes affecting isolated and particularly small populations and may lead to a loss of adaptability under extreme circumstances. The majority of the Canadian populations of *Tradescantia occidentalis* are isolated from each other, and the low levels of genetic diversity observed in Saskatchewan and Alberta indicate that genetic drift and inbreeding may be acting upon these populations. It is important to note that low levels of genetic variability do not necessarily imply that there is a lack of adaptive capability (Milligan et al. 1994).

Other factors, such as reproductive strategies like selfing or cleistogamy, may explain the low levels of genetic variation in the Saskatchewan and Alberta populations of *Tradescantia occidentalis*. It is plausible that rates of selfing may differ among the populations. Although there is no record of autogamy in this species, several members of the Commelinaceae reproduce with cleistogamous flowers (Faden 2000) or are known to be self-compatible (Hrycan and Davis 2005). Some studies suggest that members of

the genus *Tradescantia* produce sterile offspring when selfed (Sinclair 1968) and many members of the Tradescantieae tribe are self-incompatible (Owens 1981). Thus, the likelihood of autogamy in *T. occidentalis* exists, but the rates may be low and resulting offspring have the potential to be sterile.

An alternative hypothesis to explain the low levels of genetic diversity is the founder effect. If *Tradescantia occidentalis* seeds were transported by humans, intentionally or otherwise, the subsequent establishment and expansion from a few founders may account for low levels of diversity in Alberta and Saskatchewan. This idea has been used to account for low levels of diversity in other species (Husband and Barrett 1991). Founder effects, particularly in unstable or changing environments, have been reported to reduce genetic variation (Baskauf et al. 1994). However, this scenario seems unlikely as there are no records of intentional introduction of this species to these locations.

The Manitoba populations have the lowest similarity indices among the three provinces. The intrapopulation genetic similarity ranges from 0.48-1.00 in the Routledge (Fig. 5.3A-D) and Lauder populations (Fig. 5.5A-D), and 0.40-1.00 in the MHHC population (Fig. 5.4A-D). These values concur with those observed in the Meliaceae (Singh et al. 1999) and Fabaceae (Travis et al. 1996). A previous genetic diversity study on *Tradescantia hirsuticaulis* involving different molecular markers and analytical methods also reports high levels of variation (Godt and Hamrick 1993). For the Manitoba populations, it was hypothesized that individuals form genetic groups based on their geographic location. However, the dendrogram topologies (Figs. 5.3-5.6A-D) do not support this hypothesis in any of the populations, suggesting that there is a panmictic system operating in Manitoba as well.

The results of the MHHC population analysis are somewhat unusual because this population is the smallest of the five Canadian populations, and has higher genetic diversity compared to larger populations, such as those of Alberta and Saskatchewan. This disagrees with studies in the Ranunculaceae, which state that smaller populations do not maintain genetic diversity values of larger populations (Hensen et al. 2005). However, other studies involving endangered plants in the Ericaceae have shown high levels of intrapopulation variability despite small population size (Zawko et al. 2001).

### 5.4.2 Interpopulation Genetic Diversity

The majority of individuals from the Routledge, Saskatchewan, and Alberta populations form discrete clusters in the combined analysis (Fig. 5.10). The intermixing of individuals from the MHHC and Lauder populations is likely due to the ongoing process of gene flow between these populations and shared polymorphic sites that are similar in size but not necessarily homologous. According to SAHN analysis, the level of genetic similarity among the five populations is relatively high (64%) indicating that the populations have experienced low to moderate genetic differentiation. The hypothesis regarding the lack of gene flow among the populations in Alberta, Manitoba, and Saskatchewan is supported by the genetic similarity values and dendrogram topology in SAHN (Fig. 5.10), in which the majority of individuals in the populations form separate clusters. The same relationship is supported by the NJOIN analysis (Fig. 5.12 B, D).

Nei (1978) classified levels of genetic distance <0.05 as low, between 0.05 and 0.15 as medium, and >0.15 as high. According to this definition, the distances calculated from primers two and eight in SAHN (Fig. 5.11B, D) are high. As indicated,

the primer with the most expected and reasonable results is primer one, from which it can be concluded that there is some level of gene flow between MHHC and Lauder, but not among the provinces (Figs. 5.11A, 5.12A).

The relatively low levels of genetic distance between Saskatchewan and Alberta may be indicative of the historical distribution of this species and may be reflective of one or a combination of the following scenarios: 1) the Canadian populations have not been separated from the continuous distribution in the United States of America for a period long enough for a significant amount of differentiation to occur, 2) that the AFLP primers did not target sufficiently variable genomic regions to observe population differentiation, or 3) that genetic similarity is inherently high in *Tradescantia occidentalis*. It is also hypothesized based on these results that the populations of *T. occidentalis* from Alberta and Saskatchewan separated from each other more recently compared to the populations from Saskatchewan and Manitoba. Further investigation of genetic variation in this species, including specimens from populations in the United States, is required to test these hypotheses. This is especially important because it has been shown that genetic variation does not always exist between geographically restricted and widespread members of the same species (Karron et al. 1988), a geographic pattern well-suited for *T. occidentalis*.

In the Commelinaceae, a genetic diversity study of *Tradescantia hirsuticaulis* showed values of Nei's genetic distance similar to the results of this study (Godt and Hamrick 1993). As in *T. hirsuticaulis*, the lack of gene flow among *T. occidentalis* populations can be attributed to the disjunct nature of the habitat and lack of specialized pollinators. Genetic diversity studies in the Rubiaceae (Russell et al. 1999) and Cupressaceae (Wang et al. 2004) report similar values of interpopulation genetic

distance. In addition, studies in other monocots, for example the Iridaceae, found similar values of genetic distance among populations separated by large geographic distances (Arafeh et al. 2002).

### 5.4.3 Conservation Genetics and *Tradescantia occidentalis*

Even if no immediate conservation action is required, understanding the genetic structure of a population provides insight into the history of the species (Lacy 1988), and the processes of speciation and adaptation, and population dynamics (Bussell 1999). Genetic variability within populations may affect the evolutionary potential of a species (Gaudeul et al. 2000), though the extent to which low genetic variability decreases the potential of a species to adapt to environmental change has been debated (Milligan 1994). According to the results presented in this study, the low levels of genetic diversity within the Canadian populations of *Tradescantia occidentalis*, especially Saskatchewan and Alberta, are of future concern because of the potential implications regarding high genetic similarity and a potential reduced ability to adapt to environmental change. However, other estimates of genetic diversity are necessary to confirm these findings with other primers or techniques because a single type of molecular marker does not provide the best estimates of genome-wide variability in organisms (Avise 1994).

The low levels of genetic variability observed in this study may also be related to the AFLP technique, which yields fragments of the same size and are interpreted as equal, even though they may be from a different genomic regions. Thus the issue of analogy versus homology is in place (Deprés et al. 2003; Crawford and Mort 2004). Because of this, the results provided here are preliminary and should be interpreted with

some caution until additional markers are explored. It is clear, however, that each population has genetic distinctness as shown by the number of polymorphic sites. Thus, some portions of the genome are unique to each population.

Canadian populations of *Tradescantia occidentalis* are isolated from each other which means that there is no gene flow among populations across the prairie provinces. In the genetic diversity study in *T. hirsuticaulis*, gene flow estimates within each population are higher than among populations (Godt and Hamrick 1993). Though the effective population size ($N_e$) in *T. occidentalis* is unknown, it can be assumed that $N_e$ is sufficient to prevent inbreeding depression because this value is generally much smaller than the population size. This is a relevant issue because inbreeding depression can be prevented in populations with effective population sizes greater than 50 unless severe stress is applied (Lande 1999). At present, *T. occidentalis* does not appear to be at risk of inbreeding depression; however, it would be a future concern because of the relatively low levels of genetic diversity especially in the event of a dramatic decline in population number.

High genetic differentiation among populations usually indicates a lack of gene flow (Ellstrand and Elam 1993; Gaudeul et al. 2000) or a limited genetic pool due to founder effects (Husband and Barrett 1991). Given the geographic distance among the populations in Alberta, Saskatchewan and Manitoba, it is unlikely that gene flow is operating among populations in different provinces though it is possible that pollinators, for example bumble bees (*Bombus* sp. L.), may facilitate pollen transfer between the Lauder and MHHC populations within Manitoba. Correlations between genetic distance and geographic distance with gene flow have been found in the study on *T. hirsuticaulis* (Godt and Hamrick 1993). In addition, studies in the Asteraceae have indicated that

there is an increased level of divergence in range edge populations (Jump et al. 2003), which is a similar situation to the Canadian populations of *T. occidentalis*.

While *Tradescantia occidentalis* is not in immediate danger of extirpation, the understanding of the genetic structure of populations is relevant in the event that one population is eradicated. Restricted gene flow among populations may impair the future survival capability of these populations because deleterious mutations may accumulate, decreasing individual viability (Couvet 2002). According to the results of this study, in the event of population decline, transplantations of individuals from other populations should be implemented with caution because of the low to moderate level of differentiation among populations. Outbreeding depression, the mating of individuals from genetically differentiated populations, must be avoided because it results in the break up of co-adapted gene complexes or the additive effects of alleles conferring advantages under different environmental conditions (Beebee and Rowe 2004). The MHHC population is very small relative to the other populations and because they are closely related, individuals from Lauder may be used to augment the MHHC population in case it is necessary.

The *in situ* protection of the current *Tradescantia occidentalis* populations in their natural habitat remains the most viable conservation management strategy of this threatened species. This includes control of invasive plant species, monitoring grazing, and prevention of further fragmentation. It is important to consider, however, that peripheral populations like *T. occidentalis* in Canada, are small, isolated, and occur in ecologically marginal habitats and that these types of populations are subjected to more stressful conditions (Lammi et al. 1999). Habitat fragmentation in plants is further complicated because of the sessile habit of these plants and the dependence on vectors of

pollen and propagules for dispersal (Young et al. 1996). Fortunately, some of the Canadian populations are already situated in protected areas. For example, Highway 19 and Douglas are protected in the Douglas Provincial Park, and in Manitoba, MHHC is situated on Manitoba Habitat Heritage Corporation land. The populations that occur on private land are respected by the landowners and thus, at the present time, are secure.

In addition to habitat protection, *ex situ* methods of conservation management of *Tradescantia occidentalis* are recommended. Fitness components that are under selection, including germination rate, seedling growth, and rate of seed production, are important for the persistence of threatened species and therefore should be used as a priority in setting conservation management goals (Lammi et al. 1999). For example, creating a seed bank may be considered to store seeds of different genotypes in the event of a drastic population decline. Seeds should be collected from each population to maintain the genetic properties of each population. These seeds could then be grown in a greenhouse and used to augment populations. Similar management strategies have been implemented in various species (Liu et al. 2006) but its success is yet to be determined. In Saskatchewan, the Plant Gene Resources of Canada (PGRC) project has been created with the following objectives: 1) to collect seeds, including those from wild plants, to maintain genetic diversity, 2) to develop protocols for *ex situ* regeneration of wild plant species, 3) to determine seed germination and pollination requirements, and 4) to study seed longevity (PGRC 2006). A collaborative study with the PGRC would be greatly beneficial to the *ex situ* conservation of *T. occidentalis.* In all, the implementation of a seed bank will promote more research into seed viability, longevity, and germination requirements of this species, which will lead to a more comprehensive understanding of the ecological requirements of this species in Canada.

## 5.5 Concluding Remarks

Several final conclusions can be made from this research. In general, the *Tradescantia occidentalis* populations in Saskatchewan and Alberta have lower levels of intrapopulation genetic diversity than the three Manitoba populations. In addition, levels of divergence were greater across the prairie provinces than among the closely situated Manitoba populations. Overall, it can be concluded that there is no gene flow among the provinces due to geographical isolation. This is a concern for the conservation of *T. occidentalis* because it limits management strategies such as transplantation. Habitat protection is the most viable strategy for maintaining *T. occidentalis* in Canada.

Along with studies dealing with the biological aspects of this species, future molecular studies with additional markers should be undertaken to compare to the results presented here. Likewise, a comparative study of the Canadian populations in relation to populations in the United States of America is desirable to provide a better indication of the expected levels of genetic diversity in *Tradescantia occidentalis*. This study provides relevant information on the levels of genetic diversity in and among Canadian populations of *T. occidentalis* and can be used as a model for further investigation of this and other species at risk in Canada. Significant progress has been made in this study, nevertheless, more information on the pollination and reproductive biology of this species is required to fully understand pollen dispersal and viability, breeding systems, and compatibility systems leading to a more comprehensive understanding of *T. occidentalis*.

## 6. General Conclusions

This study of threatened populations of *Tradescantia occidentalis* can be used as a model for future studies on species at risk in Canada. It encompassed demographic, habitat, and plant community aspects associated with *T. occidentalis*. In addition, this research explored the application of molecular techniques to investigate levels of genetic variation and propose conservation management strategies. The main objectives were fulfilled; however, additional questions arose that need to be addressed in the future.

Population size in *Tradescantia occidentalis* has been underestimated in previous years. This is especially true in Saskatchewan and Alberta, where portions of these populations remained undiscovered until a few years ago. In Manitoba, the population sizes have remained relatively stable. It was previously thought that *T. occidentalis* populations experienced dramatic fluctuations in size from year to year. However, our results and research conducted by MCDC and PFRA illustrated that consistent sampling methods show relatively small population fluctuations. Yearly monitoring is needed to support this hypothesis; thus, long term monitoring of populations is necessary because the habitat is relatively small and restricted to fragile environments with sandy substrate.

From the molecular results, it can be concluded that the Canadian populations of *Tradescantia occidentalis* have naturally high levels of intrapopulation genetic similarity, especially in Saskatchewan and Alberta. In contrast, it appears that there may be gene flow among the Manitoba populations, in particular MHHC and Lauder, due to higher levels of genetic diversity. The Saskatchewan and Alberta populations are the

greatest in size and therefore it is unlikely that either population is seriously threatened by inbreeding depression. Based on the degree of differentiation obtained with these results, it is hypothesized that the Canadian populations of *Tradescantia occidentalis* are remnant of a larger continuous distribution and the Alberta and Saskatchewan populations appear to have been separated from the main distribution for a longer period than the Manitoba populations.

Despite the apparent stability of the populations, several factors must be considered in future conservation and biodiversity programs. Foremost, the most predominant threat to *Tradescantia occidentalis* is the presence of *Euphorbia esula* in Saskatchewan and Manitoba. As encroachment of *E. esula* continues, dune stabilization may cause a decline in *T. occidentalis* in Saskatchewan and Manitoba. However, in Alberta, *T. occidentalis* grows primarily in sandy prairie with dense vegetation, so the possibility exists that it may be able to adapt to those conditions in Saskatchewan and Manitoba. Grazing is a concern in Saskatchewan and Alberta, though current grazing regimes do not appear to have a negative impact on the Canadian populations. Petroleum exploration is a concern in Alberta because it results in loss of *T. occidentalis* habitat and has been proposed for the area containing this species.

Although all of the above factors must be considered in the conservation of *Tradescantia occidentalis*, the primary conservation strategy should be to protect its natural habitats. The significant levels of interpopulation genetic diversity suggest that efforts should be made to conserve all of the Canadian populations and habitats across the species' range. Further exploration of genetic diversity using another technique, for example microsatellites, will be instrumental in providing additional information on

genetic diversity of the Canadian populations and comparison with the genetic structure of the American populations. Transplantation of individuals to augment populations is unlikely to be successful because of population differentiation and could lead to outbreeding depression. For transplantation to be a viable option, populations would have to be genetically similar yet heterogeneous as to introduce more genetic variability. *Ex situ* methods of conservation, such as the creation of a seed bank, should be implemented prior to the event of a drastic population decline. Though the populations are of considerable size, it is not recommended to change the national or provincial rankings to vulnerable because of the limited number and isolation of the populations in this country. Decreasing its conservation priority may lead to the decline of *T. occidentalis* by promoting land use that is detrimental to this species. For example, oil and gas exploration has been considered in the location of the Alberta population, but *T. occidentalis* has been protected under Species at Risk legislation. Further research in several areas, including reproductive biology, habitat and comparison of levels of genetic diversity among populations in Canada and the United States of America is required to better understand *T. occidentalis*.

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**Appendix I:** Raw and calculated population data for the five populations of *Tradescantia occidentalis* in Canada, 2005 and comparison to previous estimates.

**Table AI.1.** Raw and calculated data for *T. occidentalis* in the PFRA pasture in Saskatchewan, 2005 (Raw data from Godwin and Sumners, unpubl.).
**Table AI.2**. Raw and calculated data for the *T. occidentalis* subpopulations, Douglas and Highway 19 (SK), 2005.
**Table AI.3.** Raw and calculated data for the *T. occidentalis* population in Routledge, MB 2005.
**Table AI.4.** Raw and calculated data for the *T. occidentalis* population in MHHC, MB 2005.
**Table AI.5.** Raw and calculated data for the *T. occidentalis* population in Lauder, MB 2005.
**Table AI.6.** Raw and calculated data for the *T. occidentalis* population in Alberta, 2005 (Data from Remarchuk 2005).
**Table AI.7** Comparison of the 2005 population estimates of *T. occidentalis* to previous estimates. Percentages represent the proportion of the maximum estimated number.

**Table AI.1.** Raw and calculated data for the *T. occidentalis* population in the PFRA pasture in Saskatchewan, 2005 (Raw data from Godwin and Sumners, unpubl.). Density values are provided in plants/$m^2$. The numbers of flowering and grazed plants and stems are not mutually exclusive.

| Polygon | Transect | No. Plants | No. Flowering Plants | No. Grazed Plants | Plant Density | Flowering Plant Density | Grazed Plant Density |
|---|---|---|---|---|---|---|---|
| North | 103N | 0 | 0 | 0 | 0 | 0 | 0 |
| | 103S | 210 | 112 | 124 | 0.1750 | 0.0933 | 0.1033 |
| | 115 | 397 | 290 | 177 | 0.3308 | 0.2416 | 0.1475 |
| | 116 | 63 | 43 | 29 | 0.0525 | 0.0358 | 0.0241 |
| | 200 | 251 | 143 | 91 | 0.2091 | 0.1191 | 0.0758 |
| | 201 | 62 | n/a | n/a | 0.0516 | n/a | n/a |
| | 202 | 157 | n/a | n/a | 0.1308 | n/a | n/a |
| | 203S | 66 | n/a | n/a | 0.0550 | n/a | n/a |
| | 203N | 63 | n/a | n/a | 0.0525 | n/a | n/a |
| **Total North** | 9 | 1,269 | 588 | 421 | n/a | n/a | n/a |
| **Avg. and S.D.** | n/a | 141 | 147 | 84 | 0.1175 ± 0.0951 | 0.0979 ± 0.0751 | 0.0701 ±0.0447 |
| South | 101 | 59 | n/a | n/a | 0.0491 | n/a | n/a |
| | 101B | 0 | n/a | n/a | n/a | n/a | n/a |
| | 101C | 0 | n/a | n/a | n/a | n/a | n/a |
| | 101D | 0 | n/a | n/a | n/a | n/a | n/a |
| **Total South** | 4 | 59 | n/a | n/a | n/a | n/a | n/a |
| **Avg.** | n/a | 15 | n/a | n/a | 0.0491 | n/a | n/a |
| **Area of Occupancy** | | | | | **Total Population Estimate** | | |
| 479,651 $m^2$ (North) 27,291 $m^2$ (South) | | | | | 56,359 ± 38,635 (North) 1,340 ± 4,261 (South) | | |

**Table AI.2**. Raw and calculated data for the *T. occidentalis* subpopulations Douglas and Highway 19 (SK), 2005. Density values are provided in stems or plants/$m^2$. The number of grazed and flowering stems and plants are not mutually exclusive.

| Polygon | Area ($m^2$) | Number | | No. Flowering | | No. Grazed | | Density | | Flowering Density | | Grazed Density | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Stems | Plants | Stems | Plants | Stems | Plants | Stems | Plants | Stems | Plants | Stems | Plants |
| Douglas | 11,887 | 696 | 430 | 634 | 422 | 17 | 9 | 0.0585 | 0.0361 | 0.0533 | 0.0355 | 0.0014 | 0.0007 |
| Hwy 19 | 5,895 | 481 | 260 | 347 | 216 | 20 | 12 | 0.0815 | 0.0366 | 0.0441 | 0.0033 | 0.0588 | 0.0020 |
| **Total** | 17,782 | 1,177 | 690 | 981 | 638 | 37 | 21 | n/a | n/a | n/a | n/a | n/a | n/a |
| **Avg.** | 8,891 | 589 | 345 | 491 | 319 | 19 | 11 | 0.0700 | 0.0364 | 0.0487 | 0.0194 | 0.0301 | 0.0013 |
| **Total Stem Estimate*** | | | | **Total Plant Estimate*** | | | | **Standard Deviation** | | | | | |
| 1,177 | | | | 690 | | | | 0.0046 | 0.0002 | 0.0046 | 0.0160 | 0.0287 | 0.0006 |

* No standard deviation is provided because the total number of stems and plants was counted instead of extrapolated.

**Table AI.3.** Raw and calculated data the for *T. occidentalis* population in Routledge, MB 2005. Density values are provided in stems or plants/$m^2$. The number of grazed stems and plants is not mutually exclusive.

| Transect | Number | | No. Flowering | | No. Grazed | | No. Pink | Density | | Flowering Density | | Grazed Density | | Pink Density |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stems | Plants | Stems | Plants | Stems | Plants | Stems | Stems | Plants | Stems | Plants | Stems | Plants | Plants |
| 1 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | 0.0222 | 0.0222 | 0.0222 | 0.0222 | 0 | 0 | 0 |
| 2 | 21 | 17 | 16 | 16 | 2 | 2 | 0 | 0.1166 | 0.0944 | 0.0944 | 0.0888 | 0.0111 | 0.0111 | 0 |
| 3 | 23 | 18 | 18 | 18 | 0 | 0 | 0 | 0.1277 | 0.1000 | 0.1000 | 0.1000 | 0 | 0 | 0 |
| 4 | 33 | 29 | 33 | 29 | 0 | 0 | 0 | 0.1833 | 0.1611 | 0.1833 | 0.1611 | 0 | 0 | 0 |
| 5 | 16 | 11 | 14 | 11 | 0 | 0 | 0 | 0.0888 | 0.0611 | 0.0777 | 0.0611 | 0 | 0 | 0 |
| 6 | 38 | 30 | 35 | 30 | 0 | 0 | 0 | 0.2111 | 0.1666 | 0.1944 | 0.1666 | 0 | 0 | 0 |
| 7 | 40 | 27 | 37 | 27 | 0 | 0 | 0 | 0.2222 | 0.1500 | 0.2055 | 0.1500 | 0 | 0 | 0 |
| 8 | 115 | 65 | 101 | 65 | 1 | 1 | 0 | 0.6388 | 0.3611 | 0.5611 | 0.3611 | 0.0055 | 0.0055 | 0 |
| 9 | 136 | 66 | 105 | 64 | 5 | 3 | 0 | 0.7555 | 0.3666 | 0.5833 | 0.3555 | 0.0277 | 0.0166 | 0 |
| 10 | 154 | 75 | 124 | 75 | 6 | 4 | 0 | 0.8555 | 0.4166 | 0.6888 | 0.4111 | 0.0333 | 0.0222 | 0 |
| 11 | 77 | 44 | 72 | 44 | 2 | 2 | 0 | 0.4277 | 0.2444 | 0.4000 | 0.2444 | 0.0111 | 0.0111 | 0 |
| 12 | 45 | 27 | 41 | 27 | 1 | 1 | 0 | 0.2500 | 0.1500 | 0.2277 | 0.1500 | 0.0055 | 0.0055 | 0 |
| 13 | 19 | 13 | 19 | 13 | 0 | 0 | 3 | 0.1055 | 0.0722 | 0.1055 | 0.0722 | 0 | 0 | 0.0166 |
| 14 | 129 | 75 | 127 | 74 | 0 | 0 | 7 | 0.7166 | 0.4166 | 0.7055 | 0.4166 | 0 | 0 | 0.0388 |
| 15 | 13 | 12 | 13 | 12 | 0 | 0 | 3 | 0.0722 | 0.0666 | 0.0722 | 0.0666 | 0 | 0 | 0.0166 |
| 16 | 32 | 21 | 30 | 21 | 0 | 0 | 8 | 0.1777 | 0.1166 | 0.1666 | 0.1166 | 0 | 0 | 0.0444 |
| 17 | 25 | 19 | 23 | 19 | 1 | 1 | 3 | 0.1388 | 0.1055 | 0.1277 | 0.1055 | 0.0055 | 0.0055 | 0.0166 |
| 18 | 68 | 45 | 60 | 43 | 7 | 6 | 4 | 0.3777 | 0.2500 | 0.3333 | 0.2388 | 0.0388 | 0.0333 | 0.0222 |
| 19 | 33 | 24 | 33 | 24 | 1 | 1 | 0 | 0.1833 | 0.1333 | 0.1833 | 0.1333 | 0.0055 | 0.0055 | 0 |
| 20 | 43 | 29 | 34 | 29 | 6 | 4 | 1 | 0.2388 | 0.1611 | 0.1888 | 0.1611 | 0.0333 | 0.0222 | 0.0055 |
| 21 | 32 | 20 | 30 | 20 | 0 | 0 | 1 | 0.1777 | 0.1111 | 0.1666 | 0.1111 | 0 | 0 | 0.0055 |
| 22 | 22 | 13 | 19 | 13 | 2 | 2 | 0 | 0.1222 | 0.0722 | 0.1055 | 0.0722 | 0.0111 | 0.0111 | 0 |
| 23 | 30 | 17 | 23 | 16 | 2 | 2 | 0 | 0.1666 | 0.0944 | 0.1277 | 0.0888 | 0.0111 | 0.0111 | 0 |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Total** | 1,148 | 701 | 1,011 | 684 | 36 | 61 | 30 | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| **Avg.** | 48 | 29 | 42 | 29 | 1.5 | 2.5 | 1.3 | 0.2657 | 0.1622 | 0.2342 | 0.1606 | 0.0083 | 0.0073 | 0.0069 |
| **Total Stem Estimate** | | | **Total Plant Estimate** | | **Area of Occupancy** | | | **Standard Deviation** | | | | | | |
| 21,948 ± 8,344 | | | 13,402 ± 4,170 | | 82,590 $m^2$ | | | 0.2341 | 0.1170 | 0.1997 | 0.1157 | 0.0120 | 0.0089 | 0.0124 |

**Table AI.4.** Raw and calculated data for the *T. occidentalis* population in MHHC, MB 2005. Density values are provided in stems or plants/$m^2$. The number of grazed stems and plants are not mutually exclusive.

| Transect | Number | | No. Flowering | | No. Grazed | | No. Pink | Density | | Flowering Density | | Grazed Density | | Pink Density |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stems | Plants | Stems | Plants | Stems | Plants | Stems | Stems | Plants | Stems | Plants | Stems | Plants | Stems |
| 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0.0055 | 0.0055 | 0.0055 | 0.0055 | 0 | 0 | 0 |
| 2 | 145 | 48 | 134 | 48 | 0 | 0 | 1 | 0.8055 | 0.2666 | 0.7444 | 0.2666 | 0 | 0 | 0.0055 |
| 3 | 15 | 7 | 11 | 7 | 2 | 2 | 0 | 0.0833 | 0.0388 | 0.0611 | 0.0388 | 0.0111 | 0.0111 | 0 |
| 4 | 33 | 6 | 16 | 4 | 17 | 3 | 1 | 0.1833 | 0.0333 | 0.1722 | 0.0222 | 0.0944 | 0.0166 | 0.0055 |
| **Total** | 194 | 62 | 162 | 60 | 19 | 5 | 2 | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| **Avg.** | 49 | 16 | 41 | 15 | 5 | 1 | 0.5 | 0.2694 | 0.0861 | 0.2458 | 0.0833 | 0.0263 | 0.0069 | 0.0027 |
| **Total Stem Estimate** | | **Total Plant Estimate** | | **Area of Occupancy** | | | | **Standard Deviation** | | | | | | |
| 2,425 ± 7,261 | | 775 ± 3,511 | | 9,000 $m^2$ | | | | 0.3158 | 0.1050 | 0.2940 | 0.1065 | 0.0395 | 0.0072 | 0.0027 |

**Table AI.5.** Raw and calculated data for *T. occidentalis* in Lauder, MB 2005. Density values are provided in stems or plants/$m^2$. The number of grazed stems and plants are not mutually exclusive.

| Transect | Number | | No. Flowering | | No. Grazed | | No. Pink | Density | | Flowering Density | | Grazed Density | | Pink Density |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stems | Plants | Stems | Plants | Stems | Plants | Stems | Stems | Plants | Stems | Plants | Stems | Plants | Stems |
| 1 | 12 | 7 | 11 | 7 | 1 | 1 | 0 | 0.0666 | 0.0388 | 0.0611 | 0.0388 | 0.0055 | 0.0055 | 0 |
| 2 | 108 | 31 | 75 | 31 | 38 | 23 | 0 | 0.6000 | 0.1722 | 0.4166 | 0.1666 | 0.2111 | 0.1277 | 0 |
| 3 | 31 | 13 | 31 | 13 | 0 | 0 | 0 | 0.1722 | 0.0722 | 0.1722 | 0.0722 | 0 | 0 | 0 |
| 4 | 22 | 8 | 9 | 6 | 5 | 3 | 1 | 0.1222 | 0.0444 | 0.0500 | 0.0333 | 0.0277 | 0.0166 | 0.0055 |
| 5 | 38 | 15 | 31 | 15 | 6 | 4 | 1 | 0.2111 | 0.0833 | 0.1722 | 0.0833 | 0.0333 | 0.0222 | 0.0055 |
| 6 | 29 | 16 | 29 | 16 | 1 | 1 | 0 | 0.1611 | 0.0888 | 0.1611 | 0.0888 | 0.0055 | 0.0055 | 0 |
| 7 | 68 | 29 | 43 | 29 | 5 | 3 | 0 | 0.3777 | 0.1611 | 0.2388 | 0.1611 | 0.0277 | 0.0166 | 0 |
| 8 | 37 | 14 | 27 | 14 | 5 | 2 | 0 | 0.2055 | 0.0777 | 0.1500 | 0.0777 | 0.0277 | 0.0111 | 0 |
| 9 | 145 | 33 | 102 | 32 | 34 | 11 | 0 | 0.8055 | 0.1833 | 0.5666 | 0.1777 | 0.1888 | 0.0611 | 0 |
| 10 | 40 | 13 | 31 | 13 | 1 | 1 | 0 | 0.2222 | 0.0722 | 0.1722 | 0.0722 | 0.0055 | 0.0272 | 0 |
| **Total** | 530 | 179 | 389 | 176 | 96 | 49 | 2 | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| **Avg.** | 53 | 18 | 39 | 18 | 10 | 5 | 0.2 | 0.2944 | 0.0994 | 0.2161 | 0.0972 | 0.0533 | 0.0373 | 0.0011 |
| **Total Stem Estimate** | | **Total Plant Estimate** | | **Area of Occupancy** | | | | **Standard Deviation** | | | | | | |
| 11,916 ± 6,796 | | 4,024 ± 1,530 | | 40,470 $m^2$ | | | | 0.2226 | 0.0501 | 0.1511 | 0.0497 | 0.0743 | 0.0178 | 0.0022 |

**Table AI.6.** Raw and calculated data for the *T. occidentalis* population in Alberta, 2005. C= Counted, E= Extrapolated. Density values are provided in stems or plants/$m^2$. The number of grazed stems and plants are not mutually exclusive. (Data from Remarchuk 2005.)

| Polygon | Area ($m^2$) | C/E | Number | | No. Flowering | | No. Grazed | | Density | | Flowering Density | | Grazed Density | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Stems | Plants | Stems | Plants | Stems | Plants | Stems | Plants | Stems | Plants | Stems | Plants |
| 1 | 271.3 | C | 92 | 39 | 84 | 39 | 1 | 1 | 0.3390 | 0.1437 | 0.3095 | 0.1437 | 0.0036 | 0.0036 |
| 2 | 384.5 | C | 1077 | 622 | 1035 | 622 | 5 | 5 | 0.2808 | 0.1622 | 0.2699 | 0.1622 | 0.0013 | 0.0013 |
| 3 | 483.7 | E | 157 | 78 | 149 | 78 | 13 | 13 | n/a | n/a | n/a | n/a | n/a | n/a |
| 4 | 323.5 | C | 68 | 38 | 67 | 38 | 2 | 2 | 0.2101 | 0.1174 | 0.2070 | 0.1174 | 0.0061 | 0.0061 |
| 5 | 39.6 | E | 13 | 6 | 12 | 6 | 1 | 1 | n/a | n/a | n/a | n/a | n/a | n/a |
| 6 | 487.2 | C | 144 | 38 | 124 | 37 | 10 | 4 | 0.2955 | 0.0779 | 0.2544 | 0.0759 | 0.0205 | 0.0082 |
| 7 | 124.4 | E | 40 | 20 | 38 | 20 | 3 | 2 | n/a | n/a | n/a | n/a | n/a | n/a |
| 8 | 170.8 | C | 83 | 24 | 70 | 24 | 5 | 3 | 0.4858 | 0.1404 | 0.4097 | 0.1404 | 0.0292 | 0.0175 |
| 9 | 10006.3 | E | 3255 | 1615 | 3089 | 1615 | 275 | 191 | n/a | n/a | n/a | n/a | n/a | n/a |
| 10 | 10925.1 | E* | 2126 | 1171 | 1963 | 1168 | 94 | 54 | 0.1955 | 0.1096 | 0.1796 | 0.1093 | 0.0085 | 0.0049 |
| 11 | 484.7 | C | 165 | 75 | 147 | 75 | 18 | 7 | 0.3403 | 0.1547 | 0.3032 | 0.1547 | 0.0371 | 0.0144 |
| 12 | 162.0 | C | 151 | 53 | 132 | 53 | 15 | 9 | 0.8644 | 0.3270 | 0.8034 | 0.3270 | 0.0925 | 0.0555 |
| 13 | 3966.1 | E | 1291 | 640 | 1224 | 640 | 109 | 76 | n/a | n/a | n/a | n/a | n/a | n/a |
| 14 | 140.9 | C | 78 | 52 | 69 | 52 | 8 | 8 | 0.5453 | 0.3688 | 0.3635 | 0.3688 | 0.0567 | 0.0567 |
| 15 | 1843.3 | C | 560 | 239 | 450 | 236 | 33 | 33 | 0.3037 | 0.1286 | 0.2440 | 0.1280 | 0.0179 | 0.0179 |
| 16 | 368.5 | E | 120 | 59 | 114 | 59 | 11 | 7 | n/a | n/a | n/a | n/a | n/a | n/a |
| 17 | 670.0 | E | 218 | 108 | 207 | 108 | 18 | 13 | n/a | n/a | n/a | n/a | n/a | n/a |
| 18 | 3617.5 | E | 1177 | 584 | 1117 | 584 | 99 | 69 | n/a | n/a | n/a | n/a | n/a | n/a |
| 19 | 13137.2 | E | 4275 | 2120 | 4056 | 2120 | 361 | 251 | n/a | n/a | n/a | n/a | n/a | n/a |
| 20 | 380.7 | C | 25 | 17 | 20 | 16 | 11 | 9 | 0.0656 | 0.0446 | 0.0525 | 0.0420 | 0.0288 | 0.0236 |
| 21 | 5471.0 | E | 1780 | 883 | 1689 | 883 | 150 | 104 | n/a | n/a | n/a | n/a | n/a | n/a |
| 22 | 2449.5 | E | 797 | 395 | 756 | 395 | 67 | 47 | n/a | n/a | n/a | n/a | n/a | n/a |
| 23 | 20210.3 | E | 6576 | 3262 | 6240 | 3262 | 432 | 386 | n/a | n/a | n/a | n/a | n/a | n/a |
| 24 | 43817.1 | E | 14258 | 7072 | 13531 | 7072 | 1204 | 837 | n/a | n/a | n/a | n/a | n/a | n/a |
| 25 | 5239.5 | E | 1704 | 846 | 1618 | 846 | 144 | 100 | n/a | n/a | n/a | n/a | n/a | n/a |
| 26 | 51027.9 | E | 16604 | 8235 | 15757 | 8235 | 1403 | 975 | n/a | n/a | n/a | n/a | n/a | n/a |
| **Total** | 7,676.8 | n/a | 3,627 | 1,861 | 2,742 | 1,523 | 203 | 135 | n/a | n/a | n/a | n/a | n/a | n/a |
| **Avg.** | 698 | n/a | 297 | 169 | 249 | 138 | 18 | 12 | 0.3254 | 0.1614 | 0.3088 | 0.1608 | 0.0275 | 0.0191 |

| Estimated Total Area | Estimated Total Stems | Estimate Total Plants | Standard Deviation | | | | | |
|---|---|---|---|---|---|---|---|---|
| 176,204 | 56,834 ± 19,188 | 28,430 ± 11,682 | 0.2034 | 0.0941 | 0.1807 | 0.0946 | 0.0259 | 0.0186 |

* In an area of 3027.9 $m^2$, 592 stems (332 plants) were counted. 544 stems (331 plants) were flowering and 94 stems (54 plants) were grazed. The density calculated for that polygon versus the average density was used for extrapolation.

**Table AI.7.** Comparison of the 2005 population estimates of *T. occidentalis* to previous estimates. Percentages represent the proportion of the maximum estimated number.

| Population | Subset | 2005 | 2004 | 2003 | 2002 | 2001 | 1990 |
|---|---|---|---|---|---|---|---|
| Saskatchewan | PFRA North* | 56,359 (99%) | 56,583 | 38,376 (68%) | n/a | n/a | n/a |
| | PFRA South* | 1,340 (27%) | 4,951 | 2,907 (58%) | n/a | n/a | n/a |
| | Douglas** | 696 (33%) | 2,137 | n/a | n/a | n/a | n/a |
| | Highway 19** | 481 (63%) | 758 | n/a | n/a | n/a | n/a |
| Routledge*** | n/a | 13,402 | n/a | n/a | n/a | 9,422 (70%) | n/a |
| MHHC*** | n/a | 775 (98%) | n/a | n/a | n/a | 787 | n/a |
| Lauder*** | n/a | 4,024 (93%) | n/a | n/a | n/a | 4,321 | n/a |
| Alberta**** | n/a | 28,430 | n/a | 7,700 | 7,450 | 7 | 210 |

* Estimates of population size are from Godwin and Sumners (unpubl.) for 2005, and from Godwin and Thorpe (2004) for 2003 and 2004.
** Estimates of population size are provided in number of stems because 2004 estimates (SERM 2005) are in stems.
*** Estimates from 2001 are from MCDC (2005).
****Estimates from 2003, 2002, 2001, and 1990 from Smith (2002).

**Appendix II**: Taxonomic list for areas with *T. occidentalis* (Habitat Type A) arranged by family, genus and species. Species indicated by (*) have been reported in previous studies but were not found in this study.

| Taxon | Common Name | Province | | |
|---|---|---|---|---|
| **Anacardiaceae** | | | | |
| *Toxicodendron radicans* (L.) Kuntze | Poison ivy | SK | MB | |
| **Asclepiadaceae** | | | | |
| *Asclepias viridiflora* Raf. var. *viridiflora* | Green milkweed | SK | | |
| *A. viridiflora* Raf. var. *linearis* (Gray) Fern. | | | MB | |
| **Asteraceae** | | | | |
| *Ambrosia psilostachya* DC. var. *coronopifolia* (T. & G.) Farw. | Perennial ragweed | | MB | |
| *Artemisia campestris* L. | Sagewort wormwood | SK | MB | AB |
| *A. cana* Pursh | Prairie sagebrush | | MB* | |
| *A. frigida* Willd. | Pasture sagewort | SK | MB | AB |
| *A. ludoviciana* Nutt. | Prairie sagewort | SK | MB | AB |
| *Crepis tectorum* L. | Annual hawksbeard | SK | | |
| *Erigeron* sp. | Fleabane | SK | | |
| *Helianthus pauciflorus* Nutt. subsp. *subrhomboideus* (Rydb.) O. Spring & E. E. Schill. | Rhombic leaved sunflower | SK | MB | AB |
| *Heterotheca villosa* (Pursh) Shinners | Golden aster | | MB | AB |
| *Lactuca biennis* (Moench) Fernald | Tall blue lettuce | SK* | | |
| *Liatris punctata* Hook. | Blazing star | SK | MB | AB |
| *Lygodesmia juncea* (Pursh) D.Don | Annual skeleton-weed | SK | MB | AB |
| *Packera paupercula* (Michx.) A. & D. Löve | Groundsel | | MB | |
| *Solidago missouriensis* Nutt. | Missouri goldenrod | SK | MB | AB |
| *S. rigida* L. | Stiff goldenrod | | MB | AB |
| *Symphyotrichum* sp. | Aster | SK | MB | |
| *S. laeve* (L.) Á. & D. Löve var. *laeve* | Smooth aster | | | AB |
| *Tragopogon dubius* Scop. | Goat's beard | SK | MB | AB |
| **Betulaceae** | | | | |
| *Alnus viridis* (Chaix) DC. subsp. *crispa* (Aiton) Turrill | Green alder | | MB | |
| **Boraginaceae** | | | | |
| *Cryptantha fendleri* (Gray) Greene | Fendler's cryptanthe | | | AB |
| *Lappula squarrosa* (Retz) Dumort | Blue-bur | SK | MB | |
| *Lithospermum incisum* Lehm. | Puccoon | SK | MB | AB |
| **Brassicaceae** | | | | |
| *Boechera holboellii* (Hornem.) A. & D. Löve | Rock cress | | MB | |
| *Descurainia pinnata* (Walter) Britton | Pinnate tansy mustard | SK* | | |
| *D. sophia* (L.) Webb ex Prantl | Flixweed | | MB | |
| *Erysimum asperum* (Nutt.) DC. | Prairie rocket | SK | | |
| *E. inconspicuum* (S. Watson) MacMill. | Small-flowered rocket | SK | MB | AB |
| *Lepidium densiflorum* Schrad. | Common peppergrass | SK | MB | AB |
| *Lesquerella arenosa* (Richarson) Rydb. | Great plains bladderpod | SK* | | |

**Appendix II Continued**.

| Taxon | Common Name | Province | | |
|---|---|---|---|---|
| **Cactaceae** | | | | |
| *Escobaria vivipara* (Nutt.) Buxbaum var. *vivipara* | Cushion cactus | SK | MB | AB |
| *Opuntia fragilis* (Nutt.) Haw. | Brittle prickly pear | SK | MB | AB |
| *O. polyacantha* Haw. | Prickly pear | SK | MB | AB |
| **Campanulaceae** | | | | |
| *Campanula rotundifolia* L. | Bluebell | | MB | |
| **Caprifoliaceae** | | | | |
| *Symphoricarpos occidentalis* Hook. | Western snowberry | SK | MB | AB |
| **Caryophyllaceae** | | | | |
| *Cerastium arvense* L. | Field chickweed | SK | MB | |
| *Moehringia lateriflora* (L.) Fenzl. | Grove sandwort | SK* | | |
| *Silene drummondii* Hook. | Campion | | | AB |
| **Chenopodiaceae** | | | | |
| *Chenopodium fremontii* S.Watson | Fremont's goosefoot | SK* | | |
| *C. leptophyllum* (Moq.) Nutt. ex S.Watson | Narrow-leaf goosefoot | SK* | | |
| *C. subglabrum* (S. Wats.) A. Nels. | Smooth goosefoot | SK | MB | AB |
| **Commelinaceae** | | | | |
| *Tradescantia occidentalis* (Britt.) Smyth | Western spiderwort | SK | MB | AB |
| **Cupressaceae** | | | | |
| *Juniperus horizontalis* Moench | Creeping juniper | SK | MB | |
| **Cyperaceae** | | | | |
| *Carex* sp. | Sedge | | MB | |
| *C. douglassii* Boott. | Douglas sedge | SK* | | |
| *C. duriuscula* C.A. Mey. | Spike-rush sedge | SK | | |
| *C. foenea* Willd. | | SK* | | |
| *C. inops* Bailey subsp. *heliophila* (Mackenzie) Crins | Sun sedge | SK | | AB |
| *C. obtusata* Lilj. | Obtuse sedge | SK* | | |
| *C. scoparia* Schkuhr. ex Willd. | Broom sedge | SK* | | |
| *Cyperus schweinitzii* Torr. | Schweinitz's sedge | SK | MB | |
| **Elaeagneaceae** | | | | |
| *Elaeagnus commutata* Bernh *ex.* Rydb. | Silverberry | SK | MB | AB |
| **Equisetaceae** | | | | |
| *Equisetum laevigatum* A. Braun | Smooth scouring rush | | MB | AB |
| **Ericaceae** | | | | |
| *Arctostaphylos uva-ursi* (L.) Spreng. | Common bearberry | SK | MB | |

**Appendix II Continued**.

| Taxon | Common Name | Province | | |
|---|---|---|---|---|
| **Euphorbiaceae** | | | | |
| *Chamaesyce serpyllifolia* (Pers.) Small subsp. *serpyllifolia* | Thyme-leaf spurge | | MB | |
| *Euphorbia esula* L. | Leafy spurge | SK | MB | |
| **Fabaceae** | | | | |
| *Astragalus* sp. | Milk vetch | SK | | |
| *Dalea candida* Michx. ex Willd. | White prairie clover | SK* | | |
| *D. purpurea* Vent. | Purple prairie clover | SK | MB | AB |
| *D. villosa* (Nutt.) Spreng. | Hairy prairie clover | SK* | MB | |
| *Glycyrrhiza lepidota* Pursh | Wild Licorice | SK | | AB |
| *Lathyrus ochroleucus* Hook. | Yellow vetchling | SK | | |
| *L. venosus* Muhl. ex Willd. | Forest pea | | MB | |
| *Melilotus officinalis* (L.) Lam. | Yellow sweet clover | | MB | |
| *Oxytropis sericea* Nutt. | Early yellow locoweed | | MB | |
| *Psoralidium lanceolatum* (Pursh) Rydb. | Scurf pea | SK | | |
| *Thermopsis rhombifolia* (Pursh) Richardson | Golden bean | SK | | AB |
| *Vicia americana* Muhl. ex Willd. | Wild vetch | SK | MB | |
| **Fagaceae** | | | | |
| *Quercus macrocarpa* Michx. | Bur oak | | MB | |
| **Liliaceae** | | | | |
| *Maianthemum stellatum* (L.) Link | Star-flowered Solomon's Seal | SK | MB | AB |
| **Nyctaginaceae** | | | | |
| *Mirabilis hirsuta* (Pursh) MacM. | Umbrellawort | SK | | |
| **Onagraceae** | | | | |
| *Oenothera nuttallii* Sweet | White evening primrose | SK | | AB |
| **Poaceae** | | | | |
| *Andropogon hallii* Hack. | Sand blue stem | | MB | |
| *Bouteloua gracilis* (Kunth) Lag. ex Griffiths | Blue grama grass | | MB | AB |
| *Bromus inermis* Leyss. | Awnless brome | | MB | |
| *Calamovilfa longifolia* (Hook.) Scribn. | Sand grass | SK | | AB |
| *Elymus canadensis* L. | Canada wild rye | SK | MB | AB |
| *E. lanceolatus* (Scribn. & J.G.Sm.) Gould subsp. *lanceolatus* | Northern wheatgrass | | | AB |
| *E. lanceolatus* (Scribn. & J.G.Sm.) Gould subsp. *psammophilus* (J.M. Gillett & H. Senn) Á. Löve | Sand dune wheatgrass | SK | | |
| *E. trachycaulus* (Link) Gould ex Shinners subsp. *subsecundus* (Link) Á. & D. Löve | Wheatgrass | SK* | | |
| *Festuca saximontata* Rydb. | Mountain fescue | SK | MB | AB |
| *Hesperostipa comata* (Trin. & Rupr.) Barkworth subsp. *comata* | Needle and thread grass | SK | MB | AB |

**Appendix II Continued**.

| Taxon | Common Name | Province | | |
|---|---|---|---|---|
| *Koeleria macrantha* (Ledeb) Schult. | June grass | SK | MB | AB |
| *Nassella viridula* (Trin.) Barkworth | Speargrass | | MB* | |
| *Pascopyrum smithii* (Rydb.) Á. Löve | Western wheat grass | SK | MB | AB |
| *Piptatherum micranthum* (Trin. & Rupr.) Barkworth | | SK* | | |
| *Poa pratensis* L. | Kentucky bluegrass | SK | MB | AB |
| *P. secunda* J. Presl. | Big bluegrass | SK* | | |
| *Sporobolus cryptandrus* (Torr.) A. Gray | Sand dropseed | SK | MB | AB |
| *S. heterolepis* (A. Gray) A. Gray | Prairie dropseed | SK* | | |
| **Polygonaceae** | | | | |
| *Polygonum aviculare* L. | Knotweed | SK* | | |
| *Rumex venosus* Pursh | Wild begonia | | | AB |
| **Primulaceae** | | | | |
| *Androsace septentrionalis* L. | Fairy candelabra | SK | | |
| **Ranunculaceae** | | | | |
| *Anemone cylindrica* A. Gray | Long-fruited anemone | | MB | |
| **Rosaceae** | | | | |
| *Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem | Saskatoon | | MB | |
| *Chamaerhodos erecta* (L.) Bunge | Little rose | SK | MB | AB |
| *Fragaria vesca* L. | Woodland strawberry | | MB | |
| *Geum triflorum* Pursh | Three-flowered avens | SK* | | |
| *Potentilla pensylvanica* L. | Prairie cinquefoil | | MB | |
| *Prunus virginiana* L. | Chokecherry | SK | MB | AB |
| *Rosa acicularis* Lindl. | Prickly rose | | | AB* |
| *R. arkansana* Porter | Prairie rose | SK | MB | AB |
| *R. woodsii* Lindl. | Wood's rose | SK* | MB* | |
| **Rubiaceae** | | | | |
| *Galium boreale* L. | Northern bedstraw | SK | MB | |
| **Salicaceae** | | | | |
| *Populus tremuloides* Michx. | Trembling Aspen | SK | | |
| *Salix* sp. | Willow | | MB | |
| *S. planifolia* Pursh | Mountain willow | | | AB |
| **Santalaceae** | | | | |
| *Comandra umbellata* (L.) Nutt. | Bastard toad-flax | SK | MB | |
| **Scrophulariaceae** | | | | |
| *Penstemon nitidus* Douglas ex Benth. | Waxleaf penstemon | SK* | | |
| **Selaginellaceae** | | | | |
| *Selaginella densa* Rydb. | Little club moss | SK | | AB |
| **Violaceae** | | | | |
| *Viola adunca* Sm. | Early blue violet | | MB | |

**Appendix III**: Taxonomic list for areas of suitable habitat without *T. occidentalis* (Habitat Type B) arranged by family, genus and species. Species indicated by (*) have been reported in previous studies but were not found in this study.

| Taxon | Common Name | Province | | |
|---|---|---|---|---|
| **Anacardiaceae** | | | | |
| *Toxicodendron radicans* (L.) Kuntze | Poison ivy | | MB | |
| **Asclepiadaceae** | | | | |
| *Asclepias viridiflora* Raf. var. *viridiflora* | Green milkweed | SK | MB | |
| *A. viridiflora* Raf. var. *linearis* (Gray) Fern. | | | MB | |
| **Asteraceae** | | | | |
| *Antennaria parvifolia* Nutt. | Small-leaf pussytoes | SK | | |
| *Artemisia campestris* L. | Sagewort wormwood | SK | MB | AB |
| *A. cana* Pursh | Prairie sagebrush | | MB* | |
| *A. frigida* Willd. | Pasture sagewort | | MB | AB |
| *A. ludoviciana* Nutt. | Prairie sagewort | SK | MB | AB |
| *Helianthus pauciflorus* Nutt. subsp. *subrhomboideus* (Rydb.) O. Spring & E. E. Schill. | Rhombic leaved sunflower | | | AB |
| *Heterotheca villosa* (Pursh) Shinners | Golden aster | SK | MB | AB |
| *Liatris punctata* Hook. | Blazing star | SK | MB | AB |
| *Lygodesmia juncea* (Pursh) D.Don | Annual skeleton-weed | SK | MB | AB |
| *Packera cana* (Hook.) W.A. Weber & A. Löve | Prairie groundsel | SK | | |
| *P. paupercula* (Michx.) A. & D. Löve | Groundsel | | MB | |
| *Solidago missouriensis* Nutt. | Missouri goldenrod | SK | MB | AB |
| *S. rigida* L. | Stiff goldenrod | | MB | AB |
| *Symphyotrichum* sp. | Aster | SK | | |
| *S. laeve* (L.) Á. & D. Löve var. *leave* | Smooth aster | | | AB |
| *Taraxacum officinale* F.H.Wigg. aggr. | Common dandelion | SK | | |
| *Townsendia exscapa* (Richardson) Porter | Stemless townsendia | | MB* | |
| *Tragopogon dubius* Scop. | Goat's beard | | MB | AB |
| **Boraginaceae** | | | | |
| *Cryptantha fendleri* (Gray) Greene | Fendler's cryptanthe | | | AB |
| *Lappula squarrosa* (Retz.) Dumort | Blue-bur | SK | MB | |
| *Lithospermum incisum* Lehm. | Puccoon | SK | MB | AB |
| **Brassicaceae** | | | | |
| *Boechera holboellii* (Hornem.) A. & D. Löve | Rock cress | SK | MB | |
| *Descurainia pinnata* (Walter) Britton | Pinnate tansy mustard | SK* | | |
| *D. sophia* (L.) Webb ex Prantl | Flixweed | | MB | |
| *Erysimum asperum* (Nutt.) DC. | Prairie rocket | SK | MB | |
| *E. inconspicuum* (S. Watson) MacMill. | Small-flowered rocket | | MB | AB |
| *Lepidium densiflorum* Schrad. | Common peppergrass | SK | MB | AB |
| *Lesquerella arenosa* (Richarson) Rydb. | Great plains baldderpod | SK* | | |
| **Cactaceae** | | | | |
| *Escobaria vivipara* (Nutt.) Buxbaum var. *vivipara* | Cushion cactus | SK | MB | AB |
| *Opuntia fragilis* (Nutt.) Haw. | Brittle prickly pear | SK | MB | AB |

**Appendix III Continued**.

| Taxon | Common Name | Province | | |
|---|---|---|---|---|
| *O. polyacantha* Haw. | Prickly pear | SK | | AB |
| **Campanulaceae** | | | | |
| *Campanula rotundifolia* L. | Bluebell | SK | MB | |
| **Caprifoliaceae** | | | | |
| *Symphoricarpos occidentalis* Hook. | Western snowberry | SK | | AB |
| **Caryophyllaceae** | | | | |
| *Cerastium arvense* L. | Field chickweed | SK | MB | |
| *Moehringia lateriflora* (L.) Fenzl. | Grove sandwort | SK* | | |
| *Silene drummondii* Hook. | Campion | | MB | AB |
| **Chenopodiaceae** | | | | |
| *Chenopodium fremontii* S.Watson | Fremont's goosefoot | SK* | | |
| *C. leptophyllum* (Moq.) Nutt. ex S.Watson | Narrow-leaf goosefoot | SK* | | |
| *C. subglabrum* (S. Wats.) A. Nels. | Smooth goosefoor | | MB | AB |
| *Salsola kali* L. | Russian thistle | | | AB |
| **Cupressaceae** | | | | |
| *Juniperus horizontalis* Moench | Creeping juniper | SK | MB | |
| **Cyperaceae** | | | | |
| *Carex* sp. | Sedge | | MB | |
| *C. douglassii* Boott. | Douglas sedge | SK* | | |
| *C. duriuscula* C.A. Mey. | Spike-rush sedge | SK | | |
| *C. filifolia* Nutt. | Threadleaf sedge | | MB | |
| *C. foenea* Willd. | | SK* | | |
| *C. inops* Bailey subsp. *heliophila* (Mackenzie) Crins | Sun sedge | SK | | AB |
| *C. obtusata* Lilj. | Obtuse sedge | SK* | | |
| *C. scoparia* Schkuhr. ex Willd. | Broom sedge | SK* | | |
| *Cyperus schweinitzii* Torr. | Schweinitz's sedge | SK | MB | |
| **Elaeagneaceae** | | | | |
| *Elaeagnus commutata* Bernh *ex.* Rydb. | Silverberry | | | AB |
| **Equisetaceae** | | | | |
| *Equisetum laevigatum* A. Braun | Smooth scouring rush | | MB | AB |
| **Ericaceae** | | | | |
| *Arctostaphylos uva-ursi* (L.) Spreng. | Common bearberry | SK | | |
| **Euphorbiaceae** | | | | |
| *Chamaesyce serpyllifolia* (Pers.) Small subsp. *serpyllifolia* | Thyme-leaf spurge | | MB | |
| *Euphorbia esula* L. | Leafy spurge | SK | MB | |
| **Fabaceae** | | | | |
| *Astragalus* sp. | Milk vetch | SK | | |

**Appendix III Continued**.

| Taxon | Common Name | Province | | |
|---|---|---|---|---|
| *Dalea candida* Michx. ex Willd. | White prairie clover | SK* | | |
| *D. purpurea* Vent. | Purple prairie clover | SK | MB | AB |
| *D. villosa* (Nutt.) Spreng. | Hairy prairie clover | | MB | |
| *Glycyrrhiza lepidota* Pursh | Wild Licorice | | | AB |
| *Lathyrus ochroleucus* Hook. | Yellow vetchling | SK | MB | |
| *Thermopsis rhombifolia* (Pursh) Richardson | Golden bean | SK | MB | AB |
| *Vicia americana* Muhl. ex Willd. | Wild vetch | SK | | |
| **Juncaceae** | | | | |
| *Juncus balticus* Willd. | Wire rush | | | AB |
| **Liliaceae** | | | | |
| *Maianthemum stellatum* (L.) Link | Star-flowered Solomon's Seal | SK | MB | AB |
| **Linaceae** | | | | |
| *Linum rigidum* Pursh | Yellow flax | | MB | |
| **Nyctaginaceae** | | | | |
| *Mirabilis hirsuta* (Pursh) MacM. | Umbrellawort | SK | | |
| **Onagraceae** | | | | |
| *Oenothera nuttallii* Sweet | White evening primrose | | | AB |
| **Poaceae** | | | | |
| *Achnatherum hymenoides* (Roem. & Schult.) Barkworth | Indian rice grass | | | AB |
| *Andropogon hallii* Hack. | Sand blue stem | | MB | |
| *Bouteloua gracilis* (Kunth) Lag. ex Griffiths | Blue grama grass | | MB | AB |
| *Bromus inermis* Leyss. | Awnless brome | | | AB |
| *Calamovilfa longifolia* (Hook.) Scribn. | Sand grass | SK | | AB |
| *Elymus canadensis* L. | Canada wild rye | SK | MB | AB |
| *E. lanceolatus* (Scribn. & J.G.Sm.) Gould subsp. *lanceolatus* | Northern wheatgrass | | | AB |
| *E. trachycaulus* (Link) Gould ex Shinners subsp. *subsecundus* (Link) Á. & D. Löve | Wheatgrass | SK* | | |
| *Festuca saximontata* Rydb. | Mountain fescue | | MB | AB |
| *Hesperostipa comata* (Trin. & Rupr.) Barkworth subsp. *comata* | Needle and thread grass | SK | MB | AB |
| *Koeleria macrantha* (Ledeb) Schult. | June grass | SK | MB | AB |
| *Pascopyrum smithii* (Rydb.) Á. Löve | Western wheat grass | | | AB |
| *Poa pratensis* L. | Kentucky bluegrass | SK | MB | AB |
| *P. secunda* J. Presl. | Big bluegrass | SK* | | |
| *Sporobolus cryptandrus* (Torr.) A. Gray | Sand dropseed | SK | MB | AB |
| *S. heterolepis* (A. Gray) A. Gray | Prairie dropseed | SK* | | |
| **Polygonaceae** | | | | |
| *Polygonum aviculare* L. | Knotweed | SK* | | |
| *Rumex venosus* Pursh | Wild begonia | | | AB |

**Appendix III continued**.

| Taxon | Common Name | Province | | |
|---|---|---|---|---|
| **Primulaceae** | | | | |
| *Androsace septentrionalis* L. | Fairy candelabra | SK | | |
| **Ranunculaceae** | | | | |
| *Anemone cylindrica* A. Gray | Long-fruited anemone | | MB | |
| **Rosaceae** | | | | |
| *Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem | Saskatoon | | MB | |
| *Chamaerhodos erecta* (L.) Bunge | Little rose | | | AB |
| *Geum triflorum* Pursh | Three-flowered avens | SK* | | |
| *Potentilla pensylvanica* L. | Prairie cinquefoil | | MB | |
| *Prunus virginiana* L. | Chokecherry | SK | MB | AB |
| *Rosa acicularis* Lindl. | Prickly rose | | | AB* |
| *R. arkansana* Porter | Prairie rose | SK | MB | AB |
| *R. woodsii* Lindl. | Wood's rose | SK* | MB* | |
| **Salicaceae** | | | | |
| *Populus deltoides* W. Bartram ex Marshall | Cottonwood | | | AB |
| *P. tremuloides* Michx. | Trembling Aspen | | | AB |
| *Salix planifolia* Pursh | Mountain willow | | | AB |
| **Santalaceae** | | | | |
| *Comandra umbellata* (L.) Nutt. | Bastard toad-flax | | MB | |
| **Scrophulariaceae** | | | | |
| *Penstemon nitidus* Douglas ex Benth. | Waxleaf penstemon | SK* | | |
| **Selaginellaceae** | | | | |
| *Selaginella densa* Rydb. | Little club moss | | MB | AB |